Journal of the National Cancer Institute **73**(5):1207-1214, November 1984.

Other support: National Cancer Institute, Veterans Administration Merit Approved Research Program and the National Foundation for Cancer Research.

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

IMMUNOLOGICAL AND CHEMICAL PROPERTIES OF MOUSE α1-PROTEASE INHIBITORS

We previously described the isolation and purification of two similar α1-protease inhibitors from mouse plasma termed α1-PI(E) and α1-PI(T) because of their respective affinities for elastase and trypsin. Some of the biochemical and immunological properties of these proteins are reported. Both are acidic glycoproteins with PI's of 4.1-4.2. The plasma half-life of each inhibitor, determined after administration of the ^{125}I-protein, is approximately 4 h both in normal mice and in mice after induction of the acute phase reaction. The two proteins have almost identical amino acid compositions and similar CNBr peptide maps. Tryptic maps, however, are considerably different. Reverse-phase chromatography separated α1-PI(E) into three distinct isoforms, each eluting with approximately 60% acetonitrile. Under these conditions α1-PI(T) shows a single peak, clearly different from those of α1-PI(E). The three α1-PI(E) isoforms have the same molecular weights on sodium dodecyl sulfate-gel electrophoresis and the same tripeptide sequence at their N-terminus, and appear to be immunologically identical. Polyclonal, monospecific antibodies to each native inhibitor, prepared in rabbits, showed no crossreactivity when tested by functional assay or crossed immunoelectrophoresis. Interestingly, each antibody recognized epitopes on the C-terminal portion of its respective antigen. These studies confirm that α1-PI(E) and α1-PI(T), although highly similar, are products of different genes. Like human α1-PI, the two mouse inhibitors are partially inactivated by mild oxidation with chloramine-T, losing all elastase inhibitor and lesser amounts of antichymotryptic and antitryptic activity. However, unlike the human protein, neither α1-PI(E) nor α1-PI(T) was found to have methionine residue at its PI site.

Nathoo, S. A. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics **246**(1):162-174, 1986.

Other support: National Science Foundation.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

THE CO-OXIDATION OF AMMONIA TO NITRITE DURING THE AEROBIC XANTHINE OXIDASE REACTION

The xanthine oxidase reaction causes a co-oxidation of NH_3 to NO_2^-, which was inhibitable by superoxide dismutase, catalase, hydroxyl radical scavengers, or by the chelating agents, desferrioxamine or diethylene triaminepentaacetic acid. Hydroxylamine was oxidized to NO_2^- much more rapidly than was NH_3, and in this case superoxide dismutase or the chelating agents inhibited but catalase or the $HO^\cdot$ scavengers did

1002319286

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

oxidation of both pyridine coenzymes. Our results indicate that vanadate stimulation of NAD(P)H oxidation by biomembranes is a consequence of vanadate stimulation of NAD(P)H or NMNH oxidation by O_2^-, rather than being due to the existence of vanadate-stimulated NAD(P)H oxidases or dehydrogenases.

Liochev, S. and *Fridovich, I.*

Archives of Biochemistry and Biophysics **250**(1):139-145, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

EFFICIENCY OF PHOTOAFFINITY LABELING DNA HOMOPOLYMERS AND COPOLYMERS WITH ETHIDIUM MONOAZIDE

Photoaffinity labeling of synthetic DNAs with ethidium monoazide was studied to determine if the efficiency of adduct formation was related to DNA sequence. Equilibrium drug binding to DNA homopolymers and copolymers was quantified by phase partition techniques. The amount of drug bound to a deoxypolymer at equilibrium was then compared to the fraction of ethidium analog covalently-linked following photoactivation at the same drug/DNA input ratio. There were significant sequence-related differences in the ability of the photoaffinity probe to label DNA covalently. The efficiency of covalent-adduct formation decreased in the order poly(dG-dC)·poly(dG-dC)>poly(dG)·poly(dC)≫poly(dA-dT)·poly(dA-dT)≥poly(dA)·poly(dT). Ethidium monoazide was about 2-fold more efficient in labeling deoxyhomopolymers and deoxycopolymers composed of G-C pairs than the A-T base counterparts. In low ionic buffers (0.015 *M* Na^+), the efficiency of photoactivation decreased with increasing ethidium monoazide concentrations. However, the base sequence effect was observed over a 40-fold range of drug concentrations. Therefore, the amount of ethidium monoazide bound to a DNA site after irradiation does not appear to represent the true affinity of the drug for that site.

Dannelly, J. M., Boyce, L. and *Gaubatz, J. W.*

Photochemistry and Photobiology **43**(1):7-11, 1986.

Other support: The Hurst Foundation and the American Heart Association.

From the Department of Biochemistry, University of South Alabama, College of Medicine, Mobile.

STIMULATION OF ACTIN SYNTHESIS BY CYTOCHALASIN D IS SPECIFIC FOR THE ISOACTINS NORMALLY EXPRESSED IN MUSCLE OR NONMUSCLE CELLS

Treatment of human muscle myotube cultures with 2 μM-cytochalasin D (CD) for 6 h stimulated synthesis of both the (muscle-specific) α-actin and the (non-muscle) β and γ-actins usually expressed by these cells. In non-muscle (HEp-2) cell cultures, CD enhanced synthesis of β and γ-actin, but did not induce synthesis of α-actin, which is

1002319290

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

PATHOGENESIS OF DESMOPLASIA. I. IMMUNOFLUORESCENCE IDENTIFICATION AND LOCALIZATION OF SOME STRUCTURAL PROTEINS OF LINE 1 AND LINE 10 GUINEA PIG TUMORS AND OF HEALING WOUNDS

The structural proteins of the scirrhous line 1 and the medullary line 10 bile duct carcinomas, both syngeneic in strain 2 Sewall-Wright inbred guinea pigs, were studied. Tumor structural proteins were compared with those of healing wounds. A provisional stromal matrix of cross-linked fibrin and fibronectin was initially deposited in both tumors and wounds and was subsequently replaced by granulation tissue containing collagen types I and III. The amounts of stromal fibrin-fibronectin and collagen were characteristic of each tumor: Line 1 contained significantly greater amounts than line 10. These differences were augmented when line 1 tumor rejection was prevented with cyclosporine, permitting time for stromal maturation. In tumors and wounds laminin and collagen type IV were found only in basement membranes. The findings suggest that 1) tumor desmoplasia is analogous to wound healing, 2) both processes involve replacement of an antecedent fibrin-fibronectin provisional matrix, 3) the extent of fibrin-fibronectin is characteristic of each tumor, and 4) tumor desmoplasia correlates with the amount of fibrin-fibronectin matrix deposited.

Dvorak, H. F. et al.

Journal of the National Cancer Institute **73**(5):1195-1205, November 1984.

Other support: National Cancer Institute and the National Foundation for Cancer Research

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston.

PATHOGENESIS OF TUMOR DESMOPLASIA. II. COLLAGENS SYNTHESIZED BY LINE 1 AND LINE 10 GUINEA PIG CARCINOMA CELLS AND BY SYNGENEIC FIBROBLASTS IN VITRO

For the investigation of the pathogenesis of desmoplasia, the capacities to synthesize collagen *in vitro* of 2 bile duct carcinomas (lines 1 and 10) of Sewall-Wright inbred strain 2 guinea pigs and of syngeneic dermal fibroblasts were studied. Line 10 cells synthesized collagen type IV as judged by sensitivity to bacterial collagenase, by immunoprecipitation, by migration of proα (IV) chains and pepsin-resistant fragments on sodium dodecyl sulfate-polyacrylamide gels, and by immunofluorescence. Line 1 cells also synthesized small amounts of collagenase-sensitive protein. Neither line 1 nor line 10 cells synthesized detectable collagen type I, III, or V. Only about 1% of [^{14}C]proline incorporated by tumor cells was found in collagenase-sensitive protein. In contrast, dermal fibroblasts synthesized 4 and 128 times as much collagenase-sensitive protein as line 10 and line 1 cells, respectively, amounting to 20% of total protein synthesized. Fibroblasts produced mostly collagen types I and III, in a ratio of 7:1, and smaller amounts of collagen type V. Thus lines 1 and 10 carcinoma cells produce primarily basement membrane collagen, whereas interstitial collagens, abundant in desmoplastic tumor stroma, are fibroblast products.

Form, D. M., VanDeWater, L., *Dvorak, H. F.*, and Smith, B. D.

1002319285

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

Brett, J. G., *Godman, G. C.*, and Miller, D. A.

TISSUE & CELL **18**(1):27-49, 1986.

Other support: National Institutes of Health and the National Cancer Institute.

From the Departments of Pathology and Human Genetics and Development, College of Physicians & Surgeons of Columbia University, New York.

CYTOCHALASIN D ALTERS THE RATE OF SYNTHESIS OF SOME HEp-2 CYTOSKELETAL PROTEINS: EXAMINATION BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

The most abundant proteins of HEp-2 cells were resolved by two-dimensional gel electrophoresis. The protein spots corresponding to several cytoskeletal proteins (vimentin, α-tubulin, β-tubulin, α-actinin, tropomyosins, and cytokeratins) were identified by comigration with protein markers or by immunological techniques.

After treatment of HEp-2 cells with 0.2 μM or 2.0 μM cytochalasin D for 20 h, radioautograms of two-dimensional gel patterns of lysates from cells pulse-labeled with [^{35}S]methionine indicated that the drug altered the rate of synthesis of some proteins. The relative rate of synthesis of the identified cytoskeletal proteins was measured. Synthesis of α-actinin, the higher-molecular-mass pair of tropomyosins and actin were similarly increased with cytochalasin D treatment, suggesting coordinate induction. Vimentin and tubulin synthesis was depressed. One cytokeratin exhibited an increase in synthesis comparable to actin, another was increased to a lesser extent and one was decreased.

Tannenbaum, J. (*Godman, G. C.*)

European Journal of Biochemistry **155**:533-542, 1986.

Other support: National Science Foundation, the Muscular Dystrophy Association and the National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

CYTOSKELETAL ORGANIZATION AFFECTS CELLULAR RESPONSES TO CYTOCHALASINS: COMPARISON OF A NORMAL LINE AND ITS TRANSFORMANT

The relationships between cytoskeletal network organization and cellular response to cytochalasin D (CD) in a normal rat fibroblast cell line (Hmf-n) and its spontaneous transformant (tHmf-e), with markedly different cytoskeletal phenotypes, were compared (using immunofluorescence, electron microscopy, and DNAse I assay for actin content). Hmf-n have prominent, polar stress fiber (SF) arrays terminating in vinculin adhesion plaques whereas tHmf-e, which are apolar, epithelioid cells with dense plasma membrane-associated actin networks, lack SF and adhesion plaques. Hmf-n exposed to CD become markedly retracted and dendritic, SF-derived actin

 1002319292

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

not. Hydrazine was not detectably oxidized to NO_2^-, and NO_2^- was not oxidized to NO_3^-, by the xanthine oxidase reaction. These results are accommodated by a reaction scheme involving (a) the metal-catalyzed production of HO· from O_2^- + H_2O_2; (b) the oxidation of H_2N^- to $H_2N^\cdot$ by $OH^\cdot$; (c) the coupling of $H_2N^\cdot$ with O_2^- to yield peroxylamine, which hydrolyzes to hydroxylamine plus H_2O_2; (d) the metal-catalyzed oxidation of $HO\text{-}NH_2$ to $HO-\overset{H}{\underset{|}{N}}^\cdot$, which couples with O_2^- to yield $HO-\overset{H}{\underset{||}{N}}-OO^-$, which finally dehydrates to yield NO_2^-.

Nagano, T. and *Fridovich, I.*

Archives of Biochemistry and Biophysics **241**(2):596-601, 1985.

Other support: U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

FURTHER STUDIES OF THE MECHANISM OF THE ENHANCEMENT OF NADH OXIDATION BY VANADATE

O_2^-, whether generated photochemically or introduced as a solution of KO_2 in a nonprotic solvent, caused rapid oxidation of NADH in the presence, but not in the absence, of vanadate. Superoxide dismutase inhibited this vanadate-stimulated oxidation of NADH, while catalase had no effect. This NADH oxidation appears to be a free-radical chain reaction whose average chain length was estimated to be 15 in the photochemical system. Vanadate stimulation of NADH oxidation by biological membranes can now be viewed as a sensitive indicator of O_2^- production by those membranes.

Liochev, S. and *Fridovich, I.*

Journal of Free Radicals in Biology & Medicine **1**:287-292, 1985.

Other support: National Science Foundation and U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

EFFECTS OF SALTS ON THE LETHALITY OF PARAQUAT

Escherichia coli suffered 95 to 100% lethality when exposed to 1.0 mM paraquat for 30 min at 37°C in aerobic nutrient broth medium but did not lose viability when the exposure was done in Vogel Bonner or tryptic soy yeast extract medium. Paraquat was, however, bacteriostatic in all of these media. Salts, added to the nutrient broth medium, protected against the lethality of paraquat, whereas sucrose did not. Salts of divalent cations were much more effective than salts of monovalent cations. Paraquat increases cyanide-resistant respiration by *E. coli*; salts added before, but not after, the paraquat diminished this effect. 2,4-Dinitrophenol similarly decreased the cyanide-resistant respiration when added before, but not after, the paraquat. The lethality imposed by paraquat correlated with the rate of cyanide-resistant respiration whether

1002319287

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

this respiration was modulated by varying salt concentration at a fixed concentration of paraquat or by varying paraquat concentration at a fixed concentration of salt. We conclude that salts or 2,4-dinitrophenol interferes with the active uptake of paraquat by *E. coli* and thus prevents its lethal effect. The salt concentrations found in a number of commonly used microbiological media are sufficient to exert this effect.

Kitzler, J. and *Fridovich, I.*

Journal of Bacteriology **167**(1):346-349, 1986.

Other support: U. S. Army Research Office and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

FREE-RADICAL CHAIN OXIDATION OF 2-NITROPROPANE INITIATED AND PROPAGATED BY SUPEROXIDE

The superoxide radical $O_2^{\cdot-}$, whether produced by the xanthine/xanthine oxidase reaction or infused as KO_2, solubilized by a crown ether in dry dimethyl sulphoxide, initiated a free-radical chain oxidation of anionic 2-nitropropane. Superoxide dismutase, but not catalase, inhibited oxidation of the nitroalkane. Xanthine oxidase suffered a syncatalytic inactivation, during the co-oxidation of 2-nitropropane, which was reversed by dialysis. Cyanide exacerbated this syncatalytic inactivation and rendered it irreversible. The frequently observed oxidations of nitroalkanes by flavoenzymes now need to be re-examined to clarify the extent to which $O_2^{\cdot-}$-initiated free-radical chain oxidation contributed to the overall nitroalkane oxidation.

Kuo, C. F., and *Fridovich, I.*

Biochemical Journal **237**:505-510, 1986.

Other support: U. S. Army Research Office and the National Science Foundation.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

IRREVERSIBLE INACTIVATION OF CATALASE BY 3-AMINO-1,2,4-TRIAZOLE

It is clear from the data presented and from the pertinent earlier literature that the results reported by Williams *et al.* (*Biochemical Pharmacology* **34**, 3386, 1985) must be dismissed as artifactual due to their failure to provide for a continuous supply of H_2O_2 during exposure of the catalase to 3-AT.

Darr, D., and *Fridovich, I.*

Biochemical Pharmacology **35**(20):3642, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

1002319288

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

protein. The possibility that the proposed repressor protein is actin and that actin may thus be a feedback inhibitor of its own synthesis is discussed.

Tannenbaum, J. *et al.* (*Godman, G. C.*)

Experimental Cell Research **160**:435-448, 1985.

Other support: National Science Foundation, Muscular Dystrophy Association and National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

RED BLOOD CELLS CONTAIN A PATHWAY FOR THE DEGRADATION OF OXIDANT-DAMAGED HEMOGLOBIN THAT DOES NOT REQUIRE ATP OR UBIQUITIN

It is generally accepted that ATP is required for intracellular protein breakdown. Reticulocytes contain a soluble ATP-dependent pathway for the degradation of highly abnormal proteins and for the elimination of certain proteins during cell maturation. Reticulocytes and erythrocytes also selectively degrade proteins damaged by oxidation. When these cells were exposed to oxidants, such as phenylhydrazine or nitrite, they showed a large increase in protein breakdown. This oxidant-induced proteolysis was not inhibited in cells depleted of ATP. However, ATP depletion did prevent the degradation of pre-existent cell proteins. In reticulocyte extracts, phenylhydrazine-treated hemoglobin is also degraded rapidly by an ATP-independent process, unlike endogenous proteins and many exogenous polypeptides. This lack of an ATP requirement means that the degradation of oxidant-damaged proteins does not require ligation to ubiquitin (even though phenylhydrazine treatment does make hemoglobin a very good substrate for ubiquitin conjugation). In many respects, the pathway for breakdown of oxidant-treated hemoglobin differs from the ATP-dependent process. The latter has a much higher activation energy than the degradation of oxidized proteins. The ATP-dependent process is inhibited by hemin, 3,4-dichloroisocoumarin, diisopropylfluorophosphate and *N*-ethylmaleimide. The ATP-independent pathway is less sensitive to *N*-ethylmaleimide, hemin, and 3,4-dichloroisocoumarin and is not affected by diisopropylfluorophosphate. In addition, only the ATP-dependent proteolytic process is inactivated by dilution or incubation at 37°C in the absence of nucleotides. Reticulocytes thus contain multiple soluble systems for degrading proteins and can rapidly hydrolyze certain types of abnormal proteins by either an ATP-independent or ATP-dependent process. Erythrocytes lack the ATP-dependent process present in reticulocytes; however, erythrocytes retain the capacity to degrade oxidant-damaged hemoglobin. These two processes probably are active in the elimination of different types of abnormal proteins.

Fagan, J. M., Waxman, L. and *Goldberg, A. L.*

The Journal of Biological Chemistry **261**(13):5705-5713, May 5, 1986.

Other support: National Institute of Neurological, Communicative Diseases and Stroke.

From the Department of Physiology and Biophysics, Harvard Medical School, Boston.

1002319295

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

aggregates form large endoplasmic masses and discrete tabular aggregates at the distal ends of retraction processes. Prolonged exposure leads to recession of process, cellular rounding, and development of large cystic vacuoles. tHmf-e cells exposed to similar doses of CD display a diagnostically different response; retraction is less drastic, cells retain broad processes containing scattered actin aggregates in discrete foci often associated with plasma membrane, large tabular aggregates are never found and processes persist throughout long exposure, vacuolation is uncommon. The CD-induced microfilamentous aggregates in Hmf-n are composed of short, kinky filament fragments forming a felt-like skein, often aggregates contain a more ordered array of roughly parallel fragments, while those of tHmf-e are very short, kinky, randomly orientated filaments imparting a distinctly granular nature to the mass. Total actin content and the amount of actin associated with detergent-resistant cytoskeletons increase following CD exposure in both cell types. Throughout exposure to CD, the actin-associated contractile proteins tropomyosin, myosin, and α-actinin co-localize within the actin aggregates in both cell types. Fodrin, the protein linking cortical actin to membrane, co-localizes with actin aggregates in tHmf-e cells and most, but not all, such aggregates in Hmf-n cells, consistent with their stress fiber derivation. Vinculin is lost from the tabular aggregates at the distal ends of retraction processes in Hmf-n cells concomitant with the fragmentation and contraction of SF. The aborized processes in both cells types contain strikingly similar axial cores of bundled vimentin filaments associated with passively compressed microtubules. The characteristic CD-induced distribution of actin filament aggregates and redistribution of vimentin in these cell types also occur when cells are allowed to respread from the rounded state in the presence of CD.

Brett, J. G. and *Godman, G. C.*

TISSUE & CELL **18**(2):175-199, 1986.

Other support: National Institutes of Health.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

STIMULATION OF ACTIN SYNTHESIS BY CYTOCHALASIN D IS SPECIFIC FOR THE ISOACTINS NORMALLY EXPRESSED IN MUSCLE OR NON-MUSCLE CELLS

Treatment of human muscle myotube cultures with 2μM-cytochalasin D (CD) for 6 h stimulated synthesis of both the (muscle-specific) α-actin and the (non-muscle) β and γ-actins usually expressed by these cells. In non-muscle (HEp-2) cell cultures, CD enhanced synthesis of β and γ-actin, but did not induce synthesis of α-actin, which is not normally present in these cells. Thus, synthesis of both muscle and non-muscle actins can be increased by CD, but enhancement of actin synthesis results from increases in the isoactins usually present, rather than induction of new isotypes. Comparison of CD-treated (fused) myotube cultures with (unfused) myoblast cultures indicated that β and γ-actin synthesis was similarly enhanced in both cultures, but that α-actin synthesis was stimulated to a greater extent in the myoblast cultures. Desmin synthesis was also stimulated in the myoblasts but not the myotubes, suggesting that the effect of CD on synthesis of these developmentally regulated cytoskeletal proteins (α-actin, desmin) might be modulated by fusion or the state of differentiation of the muscle cell.

1002319293

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

PHOSPHATE INHIBITION OF THE COPPER- AND ZINC-CONTAINING SUPEROXIDE DISMUTASE: A REEXAMINATION

Phosphate was reported to be an inhibitor of copper- and zinc-containing superoxide dismutase (SOD). Thus, SOD activity, in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), was decreased by approximately 50% when the assay was made 10 mM in phosphate, and the ionic strength was adjusted with sodium fluoride. The inhibitory effect of phosphate was attributed to the neutralization of the positive charge on the guanidino residue of Arg-141. We have reexamined the effects of phosphate inhibition of SOD and found that the enzyme has identical activity in phosphate or HEPES buffer when the ionic strength is adjusted with NaBr. The putative inhibitory effect of phosphate appears to have been due to fluoride inhibition of the superoxide generating system of xanthine/xanthine oxidase. We have confirmed this result by using a photochemical generation of O_2^-. Chemical modification of the lysine residues to homoarginines does not affect the activity of the enzyme and does not impart a phosphate sensitivity. Chemical modification with phenylglyoxal caused approximately 80% inactivation of the native enzyme and 90% inactivation of the *O*-methylisourea-modified enzyme. Our results suggest that phosphate does not inhibit the copper- and zinc-containing superoxide dismutase beyond the expectations of its effect on ionic strength.

Beyer, W. F., Wang, Y. and *Fridovich, I.*

Biochemistry **25**:6084-6088, 1986.

Other support: National Science Foundation and the U. S. Army Research Office.

From the Department of Biochemistry, Duke University Medical Center, Durham, NC.

THE VANADATE-STIMULATED OXIDATION OF NAD(P)H BY BIOMEMBRANES IS A SUPEROXIDE-INITIATED FREE RADICAL CHAIN REACTION

Rat liver microsomes catalyze a vanadate-stimulated oxidation of NAD(P)H, which is augmented by paraquat and suppressed by superoxide dismutase, but not by catalase. NADPH oxidation was a linear function of the concentration of microsomes in the absence of vanadate, but was a saturating function in the presence of vanadate. Microsomes did not catalyze a vanadate-stimulated oxidation of reduced nicotinamide mononucleotide (NMNH), but gained this ability when NADPH was also present. When the concentration of NMNH was much greater than that of NADPH a minimal average chain length could be calculated from 1/2 the ratio of NMNH oxidized per NADPH added. The term chain length, as used here, signifies the number of molecules of NMNH oxidized per initiating event. Chain length could be increased by increasing [vanadate] and [NMNH] and by decreasing pH. Chain lengths in excess of 30 could easily be achieved. The K_m for NADPH, arrived at from saturation of its ability to trigger NMNH oxidation by microsomes in the presence of vanadate, was 1.5 μM. Microsomes or the outer mitochondrial membrane were able to catalyze the vanadate-stimulated oxidation of NADH or NADPH but only the oxidation of NADPH was accelerated by paraquat. The inner mitochondrial membrane was able to cause the vanadate-stimulated oxidation of NAD(P)H and in this case paraquat stimulated the

1002319289

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

THE CHEMICAL SYNTHESIS OF HIGH SPECIFIC-ACTIVITY (^{35}S) ADENOSYLHOMOCYSTEINE

The study of the family of transmethylases, critical to normal cellular function and often altered in cancer, can be facilitated by the availability of a high specific-activity *S*-adenosylhomocysteine. We report the two-step preparation of [^{35}S]adenosylhomocysteine from [^{35}S]methionine at a specific activity of 1420 Ci/mmol in an overall yield of 24% by a procedure involving demethylation of the [^{35}S]methionine to [^{35}S]homocysteine followed by condensation with 5′-chloro-5′-deoxyadenosine. The ease of the reactions, ready availability and low cost of the reagents and high specific-activity and stability of the product make the procedure an attractive one with many uses, and superior to current methodology.

Stern, P. H. and *Hoffman, R. M.*

Analytical Biochemistry **158**:408-412, 1986.

Other support: National Institutes of Health.

From the Department of Pediatrics, University of California, San Diego, La Jolla.

ORAL NICOTINE INDUCES AN ATHEROGENIC LIPOPROTEIN PROFILE

Male squirrel monkeys were used to evaluate the effect of chronic oral nicotine intake on lipoprotein composition and metabolism. Eighteen yearling monkeys were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine primates given liquid diet supplemented with nicotine at 6 mg/kg body wt day. Animals were weighed biweekly, plasma lipid, glucose, and lipoprotein parameters were measured monthly, and detailed lipoprotein composition, along with postheparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activity, was assessed after 24 months of treatment. Although nicotine had no effect on plasma triglyceride or high density lipoproteins (HDL), the alkaloid caused a significant increase in plasma glucose, cholesterol, and low density lipoprotein (LDL) cholesterol plus protein while simultaneously reducing the HDL cholesterol/plasma cholesterol ratio and animal body weight. Levels of LDL precursors, very low density (VLDL) and intermediate density (IDL) lipoproteins, were also lower in nicotine-treated primates while total postheparin lipase (LPL + HTGL) activity was significantly elevated. Our data indicate that long-term consumption of oral nicotine induces an atherogenic lipoprotein profile (↑ LDL, ↓ HDL/total cholesterol ratio) by enhancing lipolytic conversion of VLDL to LDL. These results have important health implications for humans who use smokeless tobacco products or chew nicotine gum for prolonged periods.

Cluette-Brown, J., Mulligan, J., Doyle, K., Hagan, S., Osmolski, T., and *Hojnacki, J.*

Proceedings of the Society for Experimental Biology and Medicine **182**(3):409-413, 1986.

From the Department of Biological Sciences, Graduate Biochemistry Program, University of Lowell, Lowell, MA.

10023192

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

sites. The results are consistent with the earlier observation that some antibodies prepared against nAcChoR from peripheral tissues recognize rat brain high-affinity α-bungarotoxin binding sites. The results also suggest the existence of nAcChoR-like entities in brain that do not bind toxin with a high affinity.

Lukas, R. J.

Cellular and Molecular Neurobiology **6**(3):281-291, 1986.

Other support: National Institutes of Health, Epilepsy Foundation of America, Epi-Hab Phoenix, and the Men's and Women's Boards of the Barrow Neurological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

CHARACTERIZATION OF CURAREMIMETIC NEUROTOXIN BINDING SITES ON MEMBRANE FRACTIONS DERIVED FROM THE HUMAN MEDULLOBLASTOMA CLONAL LINE, TE671

Studies were conducted on curaremimetic neurotoxin binding to the nicotinic acetylcholine receptor present on membrane fractions derived from the human medulloblastoma clonal line, TE671. High-affinity binding sites (K_D = 2 n*M* for 1-h incubation at 20°C) and low-affinity binding sites (K_D = 40 n*M*) for ^{125}I-labeled α-bungarotoxin are present in equal quantities (60 fmol/mg membrane protein). The kinetically determined dissociation constant for high-affinity binding of toxin is 0.56 n*M* (k_1 = $6.3 \cdot 10^{-3}$ min^{-1} nM^{-1}; k_{-1} = $3.5 \cdot 10^{-3}$ min^{-1}) at 20°C. Nicotine, *d*-tubocurarine, and acetylcholine are among the most effective inhibitors of high-affinity toxin binding. The quantity of toxin binding sites and their affinity for cholinergic agonists are sensitive to reduction, alkylation, and/or oxidation of membrane sulfhydryl residues. High-affinity toxin binding sites that have been subjected to reaction with the sulfhydryl reagent dithiothreitol are irreversibly blocked by the nicotinic receptor affinity reagent bromoacetylcholine. High-affinity toxin binding is inhibited in the presence of either of two polyclonal antisera or a monoclonal antibody raised against nicotinic acetylcholine receptors from fish electric tissue. Taken together, these results indicate that curaremimetic neurotoxin binding sites on membrane fractions of the TE671 cell line share some properties with nicotinic acetylcholine receptors of peripheral origin and with toxin binding sites on other neuronal tissues.

Lukas, R. J.

Journal of Neurochemistry **46**(6):1936-1941, 1986.

Other support: Men's and Women's Boards of the Barrow Neurological Foundation, Epi-Hab Phoenix and the National Institutes of Health.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

CHARACTERIZATION OF CURAREMIMETIC NEUROTOXIN BINDING SITES ON CELLULAR MEMBRANE FRAGMENTS DERIVED FROM THE RAT PHEOCHROMOCYTOMA PC12

Studies were conducted on the properties of ^{125}I-labeled α-bungarotoxin binding

1002319299

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

subunits by cAMP-dependent protein kinase increases the rate of the rapid desensitization of the receptor, a process by which the receptor is inactivated in the presence of acetylcholine (ACh). These results provide the first direct evidence that phosphorylation of an ion channel protein modulates its function and suggest that phosphorylation of postsynaptic receptors in general may play an important role in synaptic plasticity.

Huganir, R. L. et al.

Nature **321**(6072):774-776, 1986.

Other support: National Science Foundation.

From the Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, and Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY.

INTERACTIONS OF POLYCLONAL ANTI-*ELECTROPHORUS* NICOTINIC RECEPTOR ANTISERA WITH *TORPEDO* NICOTINIC RECEPTOR

Polyclonal antisera raised against solubilized and purified nicotinic acetylcholine receptor from *Electrophorus* electroplax and a polyclonal anti-α-bungarotoxin antiserum have been studied by the use of four different radioimmunoassay protocols. The results indicate unique sensitivities of different assay techniques in analysis of antibody-antigen interactions, and serve as a model for immunological study of other integral membrane proteins.

Lukas, R. J.

International Journal of Biochemistry **18**(7):609-615, 1986.

Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, Inc., and the Men's and Women's Boards of the Barrow Neurological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

INTERACTIONS OF ANTINICOTINIC ACETYLCHOLINE RECEPTOR ANTIBODIES WITH RAT BRAIN AND MUSCLE ANTIGENIC DETERMINANTS

Studies were performed to determine whether antibodies prepared against nicotinic acetylcholine receptors (nAcChoR) from electric tissue are reactive toward nAcChoR-like antigenic determinants in rat brain. Reference experiments involved the use of *Torpedo* electroplax and rat innervated muscle as tissue controls and an anti-α-bungarotoxin antiserum as a probe for curaremimetic neurotoxin binding sites. As evidenced by their ability to inhibit immunoprecipitation of *Torpedo* nAcChoR, brain or muscle membranes specifically interact with polyclonal antisera raised against *Electrophorus* electroplax nAcChoR. When the extent of polyclonal anti-nAcChoR antibody binding to muscle membranes is measured by protein A binding protocols, receptor-like antigenic determinants and toxin binding sites are found to be present in approximately equal quantities. In contrast, nAcChoR-like antigenic determinants on rat brain membranes are present at concentrations in excess of those of toxin binding

1002319298

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

ORAL NICOTINE IMPAIRS CLEARANCE OF PLASMA LOW DENSITY LIPOPROTEINS

The effect of chronic oral nicotine intake on plasma low density lipoprotein (LDL) clearance, lipid transfer protein, and lecithin:cholesterol acyltransferase (LCAT) was examined in male atherosclerosis susceptible squirrel monkeys. Eighteen yearling primates were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine monkeys given liquid diet supplemented with nicotine at 6 mg/kg body wt/day for a two-year period. Averaged over 24 months of treatment, animals in the Nicotine group had significantly higher levels of plasma and LDL cholesterol compared to Controls while plasma LCAT activity was similar for both groups. Following simultaneous injection of ^{3}H LDL and ^{14}C high density lipoprotein (HDL) cholesterol ester (CE), removal of the latter was not altered by oral nicotine while plasma clearance of ^{3}H LDL was dramatically delayed in Nicotine monkeys. Transfer of ^{14}C HDL CE to very low density lipoprotein (VLDL)-LDL particles was greatly accelerated in the Nicotine group *vs* Controls while the reciprocal movement of ^{3}H LDL CE to HDL was only higher in experimental animals at two time points following injection of the isotopes. Results from this study provide evidence that one major detrimental effect of long-term oral nicotine use is an increase in the circulating pool of atherogenic LDL which is due to: 1) accelerated transfer of lipid from HDL; and 2) impaired clearance of LDL from the plasma compartment. Diminished removal of LDL is of particular importance because an extended residence time of these particles in circulation would increase the likelihood of their deposition in the arterial wall.

Hojnacki, J. et al.

Proceedings of the Society for Experimental Biology and Medicine **182**(3):414-418, 1986.

From the Department of Biological Sciences, Graduate Biochemistry Program, University of Lowell, Lowell, MA.

PHOSPHORYLATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR REGULATES ITS RATE OF DESENSITIZATION

Recent studies have provided evidence for a role of protein phosphorylation in the regulation of the function of various potassium and calcium channels. As these ion channels have not yet been isolated and characterized, it has not been possible to determine whether phosphorylation of the ion channels themselves alters their properties or whether some indirect mechanism is involved. In contrast, the nicotinic acetylcholine receptor, a neurotransmitter-dependent ion channel, has been extensively characterized biochemically and has been shown to be directly phosphorylated. The phosphorylation of this receptor is catalysed by at least three different protein kinases (cyclic AMP-dependent protein kinase, protein kinase C and a tyrosine-specific protein kinase) on seven different phosphorylation sites. However, the functional significance of phosphorylation of the receptor has been unclear. We have now examined the functional effects of phosphorylation of the nicotinic acetylcholine receptor by cAMP-dependent protein kinase. We investigated the ion transport properties of the purified and reconstituted acetylcholine receptor before and after phosphorylation. We report here that phosphorylation of the nicotinic acetylcholine receptor on the γ- and δ--

1002319297

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

ISOLATION, GROWTH REQUIREMENTS, CLONING, PROSTACYCLIN PRODUCTION AND LIFE-SPAN OF HUMAN ADULT ENDOTHELIAL CELLS IN LOW SERUM CULTURE MEDIUM

Endothelial cells from autopsy and biopsy specimens from a variety of adult human vascular tissue were harvested by collagenase treatment and gentle swabbing of the lumenal surface. Nutrient medium MCDB 107 containing a partially purified brain-derived growth factor (5 μg/ml), epidermal growth factor (10 ng/ml) and only 2% (v/v) fetal bovine serum supported clonal and long-term serial culture (17.6 to 26.1 cumulative population doublings) of endothelial cells from vena cava, thoracic aorta and tibial arteries at a 70% rate of success. Cumulative doublings of the cell population from eight cultures were inversely proportional to age of donor of the vascular tissue from which cells were isolated. Heparin had an enhancing effect on cell growth that varied with cell strain. Prostacyclin production of human adult endothelial cell cultures was stimulated by arachidonate and thrombin by 17- to 20-fold, and 2- to 3-fold, respectively. Endogenous and stimulated rates of prostacyclin production by human adult endothelial cells were 2 to 3 times that of human adult smooth muscle cells and 20 to 30 times that of human fibroblasts.

Hoshi, H., and *McKeehan, W. L.*

In vitro Cellular & Developmental Biology **22**(1):51-56, 1986.

Other support: U. S. Public Health Service.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

CHARACTERIZATION OF MULTIPLE FORMS OF PROSTATROPIN (PROSTATE EPITHELIAL CELL GROWTH FACTOR) FROM BOVINE BRAIN

Two molecular forms of prostatropin distributed among five chromatographic peaks have been isolated from bovine brain by heparin-Sepharose affinity and reverse phase high performance liquid chromatography. One form has an apparent molecular weight of 16000 and an amino terminal sequence of asn-tyr-lys-lys-pro-lys-leu-leu-tyr-x-ser-asn-gly. The other form has an apparent molecular weight of 18000 and a blocked amino terminus. Both forms are similar in amino acid composition. The sequence of a proteolytic fragment from the blocked form overlaps the NH_2-terminal sequence of the unblocked form, therefore, the smaller form may be derived from the larger form through proteolytic processing. Both forms contain regions identical in sequence to brain-derived, heparin-binding growth factors that have been isolated on the basis of mitogenic activity for fibroblasts and endothelial cells. Both forms of prostatropin exhibit potent mitogenic activity for normal and tumor prostate epithelial cells.

Crabb, J. W., Armes, L. G., Johnson, C. M., and *McKeehan, W. L.*

Biochemical and Biophysical Research Communications **136**(3):1155-1161, May 14, 1986.

Other support: National Cancer Institute.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

1002319301

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

sites on cellular membrane fragments derived from the PC12 rat pheochromocytoma. Two classes of specific toxin binding sites are present at approximately equal densities (50 fmol/mg of membrane protein) and are characterized by apparent dissociation constants of 3 and 60 n*M*. Nicotine and *d*-tubocurarine are among the most potent inhibitors of high-affinity toxin binding. The affinity of high-affinity toxin binding sites for nicotinic cholinergic agonists is reversibly or irreversibly decreased, respectively, on treatment with dithiothreitol or dithiothreitol and *N*-ethylmaleimide. The nicotinic receptor affinity reagent bromoacetylcholine irreversibly blocks high-affinity toxin binding to PC12 cell membranes that have been treated with dithiothreitol. Two polyclonal antisera raised against the nicotinic acetylcholine receptor from *Electrophorus electricus* inhibit high-affinity toxin binding. These detailed studies confirm that curaremimetic neurotoxin binding sites on the PC12 cell line are comparable to toxin binding sites from neural tissues and to nicotinic acetylcholine receptors from the periphery. Because toxin binding sites are recognized by anti-nicotinic receptor antibodies, the possibility remains that they are functionally analogous to nicotinic receptors.

Lukas, R. J.

Journal of Neurochemistry **47**:1768-1773, 1986.

Other support: Men's and Women's Boards of the Barrow Neurological Foundation, Epi-Hab Phoenix and the National Institutes of Health.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

INTERACTIONS OF ANTI-NICOTINIC ACETYLCHOLINE RECEPTOR ANTIBODIES AT α-BUNGAROTOXIN BINDING SITES ACROSS SPECIES AND TISSUES

Two antisera prepared against the nicotinic acetylcholine receptor (nAcChoR) from *Electrophorus* exhibit comparable ability to inhibit high-affinity α-bungarotoxin binding to membrane fractions from rat brain or muscle, PC12 or TE671 cells, or *Torpedo* electric tissue. Only one of several monoclonal antibodies raised against nAcChoR from *Torpedo* inhibits toxin binding to membranes from rat brain or muscle or TE671 cells, but is considerably more potent as an inhibitor of toxin binding to *Torpedo* nAcChoR. These results indicate that some antibodies prepared against nAcChoR from electric tissue recognize epitopes near the high-affinity toxin binding sites. Some of these toxin binding site epitopes are preserved across species and tissue. The positive outcome of this study supports the continued use of toxin as a probe for at least a subset of mammalian neuronal nAcChoR.

Lukas, R. J.

Molecular Brain Research **1**:119-125, 1986.

Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, and the Men's and Women's Boards of the Barrow Neurological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

1002319300

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

ectodermal cell mitogen is also an epithelial cell growth factor that may be involved in support of prostate hyperplasia and adenocarcinoma.

Crabb, J. W., Armes, L. G., Carr, S. A., and *McKeehan, W. L.*

Biochemistry **25**:4988-4993, 1986.

Other support: National Institutes of Health.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

ROLE OF LIPOPROTEINS IN GROWTH OF HUMAN ADULT ARTERIAL ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN LOW LIPOPROTEIN-DEFICIENT SERUM

Recently improved culture conditions for human adult arterial endothelial and smooth muscle cells from a wide variety of donors have been used to study the effects of lipoproteins on proliferation of both cell types in low serum culture medium. Optimal growth of endothelial and smooth muscle cells in an optimal nutrient medium (MCDB 107) containing epidermal growth factor, a partially purified fraction from bovine brain, and 1% (v/v) lipoprotein-deficient serum was dependent on either high- or low-density lipoprotein. High- and low-density lipoprotein stimulated cell growth by three- and five-fold, respectively, over a 6-day period. Optimal stimulation of both endothelial and smooth muscle cell growth occurred between 20 and 60 μg/ml of high- and low-density lipoproteins, respectively. No correlation between the activation of 3-hydroxyl-3-methylglutaryl coenzyme A reductase activity and lipoprotein-stimulated cell proliferation was observed. Lipid-free total apolipoproteins or apolipoprotein C peptides from high-density lipoprotein were partially effective and together with oleic acid effectively replaced native high-density lipoprotein for the support of endothelial cell growth. In contrast, apolipoproteins or apolipoprotein C peptides from high-density lipoprotein alone or with oleic acid had no effect on smooth muscle cell proliferation. The results suggest a functional role of high- and low-density lipoproteins and apolipoproteins in the proliferation of human adult endothelial and smooth muscle cells.

Chen, J.-K., Hoshi, H., McClure, D. B., and *McKeehan, W. L.*

Journal of Cellular Physiology **129**:207-214, 1986.

Other support: National Cancer Institute and the National Institutes of Health.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

EFFECT OF CYANIDE ON NITROVASODILATOR-INDUCED RELAXATION, CYCLIC GMP ACCUMULATION AND GUANYLATE CYCLASE ACTIVATION IN RAT AORTA

The effects of sodium cyanide on relaxation, increases in cyclic GMP accumulation and guanylate cyclase activation induced by sodium nitroprusside and other nitrovasodilators were examined in rat thoracic aorta. Cyanide abolished nitroprusside-induced relaxation and the associated increase in cyclic GMP levels. Basal levels of cyclic GMP and cyclic AMP were also depressed. Reversal of nitroprusside-induced relaxation by cyanide was independent of the tissue level of cyclic GMP prior to

2002313303

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

SOLUBLE GUANYLATE CYCLASE FROM RAT LUNG EXISTS AS A HETERODIMER

The soluble form of guanylate cyclase (EC 4.6.1.2) from rat lung has been purified to homogeneity by one-step immunoaffinity chromatographic procedure. The purified soluble guanylate cyclase has specific activities of 432 and 49.1 nmol of cyclic GMP formed per min/mg protein with manganese and magnesium ions as a cofactor, respectively. This represents a purification of approximately 2,000-fold with a 50% recovery. The native enzyme has a molecular weight of 150,000 and a Stokes radius of 4.8 nm as determined on Spherogel TSK-G3000SW gel permeation chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis results in two protein-staining bands with molecular weights of 82,000 and 70,000. The purified soluble guanylate cyclase was also subjected to native polyacrylamide gel electrophoresis, isoelectric focusing electrophoresis, ion exchange chromatography, and GTP-agarose affinity chromatography. These additional purification procedures confirmed the presence of a single protein peak coincident with enzyme activity. The two subunits separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis were shown to have different primary structures by immunoblotting with monoclonal and polyclonal antibodies prepared against purified soluble guanylate cyclase and by peptide mapping with papain or *Staphylococcus aureus* V8 protease treatment. The data demonstrate that soluble guanylate cyclase purified from rat lung is a heterodimer composed of 82,000- and 70,000-dalton subunits with different primary structures.

Kamisaki, Y., Saheki, S., Nakane, M., Palmieri, J. A., Kuno, T., Chang, B. Y., Waldman, S. A. and *Murad, F.*

The Journal of Biological Chemistry **261**(16):7236-7241, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

INTESTINAL RECEPTOR FOR HEAT-STABLE ENTEROTOXIN OF *ESCHERICHIA COLI* IS TIGHTLY COUPLED TO A NOVEL FORM OF PARTICULATE GUANYLATE CYCLASE

A novel form of particulate guanylate cyclase tightly coupled by cytoskeletal components to receptors for heat-stable enterotoxin (ST) produced by *Escherichia coli* can be found in membranes from rat intestinal mucosa. Intestinal particulate guanylate cyclase was resistant to solubilization with detergent alone, with only 30% of the total enzyme activity being extracted with Lubrol-PX. Under similar conditions, 70% of this enzyme was solubilized from rat lung membranes. The addition of high concentrations of sodium chloride to the extraction buffer resulted in greater solubilization of particulate guanylate cyclase from intestinal membranes. Although extraction of intestinal membranes with detergent and salt resulted in greater solubilization of guanylate cyclase, a small fraction of the enzyme activity remained associated with the particulate fraction. This activity was completely resistant to solubilization with a variety of

1002313307

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

Transactions of the Association of American Physicians XCVIII, pps. 243-252, 1985.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

AMINOPHYLLINE POTENTIATES SODIUM NITROPRUSSIDE-INDUCED HYPOTENSION IN THE DOG

The biochemical mechanisms by which nitroso-vasodilators cause smooth muscle relaxation remain controversial. One theory states that the effects of nitroso-vasodilators are mediated by increased intracellular levels of cyclic GMP due to activation of guanylate cyclase. To test this hypothesis, the authors examined the effects of sodium nitroprusside (SNP) in anesthetized dogs with and without pretreatment with the phosphodiesterase inhibitor aminophylline. Aminophylline pretreatment resulted in a 2.8-fold potentiation of the hypotensive effects of a continuous infusion of SNP. Potentiation also was seen for the effects of SNP on stroke volume, heart rate, and plasma cyclic GMP levels. These results support the hypothesis that nitroso-vasodilators exert their effects via guanylate cyclase activation. The authors advise caution when vasodilator therapy with agents such as SNP, nitroglycerin, or hydralazine is instituted in patients receiving aminophylline and when aminophylline is either instituted in patients receiving aminophylline and when aminophylline is either instituted or discontinued in patients on vasodilator therapy.

Pearl, R. G., Rosenthal, M. H., *Murad, F.*, and Ashton, J. P. A.

Anesthesiology **61**(6):712-715, 1984.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Anesthesia and Medicine, Stanford University Medical Center, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

ENDOTHELIUM-DEPENDENT AND NITROVASODILATOR-INDUCED ACTIVATION OF CYCLIC GMP-DEPENDENT PROTEIN KINASE IN RAT AORTA

Cyclic GMP-dependent protein kinase (cyclic GMP-kinase) activity in isolated strips of rat aorta was measured in the absence and presence of exogenous cyclic GMP (2 μM) and expressed as a ratio. This activity ratio represented an estimate of the endogenous activation state of the enzyme. Acetylcholine (10 μM), an endothelium-dependent vasodilator, increased the activity ratio from a control value of 0.42 to 0.71 in aorta with endothelium intact. With endothelium removed, acetylcholine had no effect on cyclic GMP-kinase activity. The nitrovasodilator sodium nitroprusside (50 nM) increased activity ratios in aorta both with (0.42 to 0.54) and without (0.29 to 0.40) endothelium. Since activity ratios were higher in aortas with an intact endothelium, a tonic influence of the endothelium on aorta cyclic GMP-kinase is suggested. The vasodilator isoproterenol (3μM) had no effect on cyclic GMP-kinase activity ratios. The increases in cyclic GMP-kinase activity caused by sodium nitroprusside

1002319305

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

and probably hydroxyl free radical and some porphyrins. On the other hand, the particulate isoenzyme can be activated with agents such as *Escherichia coli* heat-stable enterotoxin, atriopeptins, and hemin. Cations, thiols, other redox agents, and detergents also have complex effects on the activity of both isoenzymes.

Murad, F.

Journal of Clinical Investigation **78**:1-5, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CHARACTERIZATION OF THE RECEPTOR FOR HEAT-STABLE ENTEROTOXIN FROM *ESCHERICHIA COLI* IN RAT INTESTINE

The receptor for the heat-stable enterotoxin (ST) from *Escherichia coli* was solubilized with Lubrol-PX from rat intestinal brush-border membranes and characterized. The binding kinetics and analog specificity of the solubilized receptor were virtually identical to those obtained with the membrane-bound receptor. Furthermore, the regulation of the receptor's affinity by cations was also maintained after solubilization, indicating a conservation of the toxin-binding site after removal of the receptor from its membrane environment. Gel filtration and sucrose density gradient sedimentation studies gave a Stokes radius of 5.5 nm and a sedimentation coefficient of 7.0 S for the solubilized receptor. The isoelectric point of the receptor was determined as 5.5 using Sephadex isoelectric focusing electrophoresis. In all of these separation techniques, the ST receptor showed a single peak of activity that was clearly separated from that of guanylate cyclase. When ^{125}I-ST was cross-linked to brush-border membranes with disuccinimidyl suberate, the affinity-labeled receptor solubilized with 0.1% Lubrol-PX eluted at a similar position as the native receptor on gel filtration chromatography. Analysis of the affinity-labeled receptor by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of reducing agent and by autoradiography revealed the presence of three specifically labeled polypeptides with apparent molecular weights of 80,000, 68,000, and 60,000. These results suggest that the ST receptor is solubilized by Lubrol-PX in an active form with preservation of its regulation by cations. Also, the ST receptor is separable from particulate guanylate cyclase indicating that the receptor is coupled to the activation of guanylate cyclase by an as yet undefined mechanism. Three subunit peptides may constitute a binding region of the receptor.

Kuno, T., Kamisaki, Y., Waldman, S. A., Gariepy, J., Schoonik, G., and *Murad, F.*

The Journal of Biological Chemistry **261**(3):1470-1476, 1986.

Other support: National Insitutes of Health and the Veterans Administration.

From the Departments of Medicine, Pharmacology, and Microbiology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

1002319309

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

TWO APPARENT HUMAN ENDOTHELIAL CELL GROWTH FACTORS FROM HUMAN HEPATOMA CELLS ARE TUMOR-ASSOCIATED PROTEINASE INHIBITORS

Two polypeptides from secretory products of human hepatoma cells were isolated and characterized on the basis of their stimulation of maintenance and growth of human endothelial cells in serum-free cell culture. Both factors were purified to homogeneity by a combination of reverse-phase, ion exchange, and molecular filtration high performance liquid chromatography. One factor (endothelial cell growth factor (ECGF-2a)) had $M_r \sim 6,500$ and pI near 6. The second (ECGF-2b) had $M_r = 27,000$ and a pI below 4.0. Both ECGF-2a and ECGF-2b exhibited single NH_2-terminal sequences. The first 25 NH_2-terminal residues of ECGF-2a and the first 49 residues of ECGF-2b were determined by gas-phase microsequencing. All clearly determined residues of ECGF-2a were identical with human pancreatic secretory trypsin inhibitor. All assignable residues of ECGF-2b were identical with urinary glycoprotein proteinase inhibitor (HI-30/EDC1). Both proteins are absent or at low levels in normal plasma and urine, but appear during acute inflammatory disease and cancer. Amino acid composition of ECGF-2a and ECGF-2b was also similar to human pancreatic secretory inhibitor and HI-30/EDC1, respectively. Both ECGF-2a and ECGF-2b inhibited bovine pancreatic trypsin (2 μg/ml) by 50% at 750 ng/ml. ECGF-2a and ECGF-2b stimulated endothelial cell number at a half-maximal dose of 50 ng/ml (8 nM) and 80 to 130 ng/ml (5 to 9 nM) protein, respectively. When assayed under identical conditions, no effect of either factor on human smooth muscle cells, human hepatoma cells, or human, rat, and mouse fibroblasts could be detected.

McKeehan, W. L. et al.

The Journal of Biological Chemistry **216**(12):5378-5383, 1986.

Other support: United States Public Health Service.

From the W. Alton Jones Cell Science Center, Lake Placid, NY.

COMPLETE PRIMARY STRUCTURE OF PROSTATROPIN, A PROSTATE EPITHELIAL CELL GROWTH FACTOR

Bovine brain prostatropin is a potent and essential mitogen for prostate epithelial cell growth. The major form of prostatropin contains 154 amino acid residues in a single amino terminally blocked chain corresponding to a molecular weight of 17400. The amino acid sequence of the 150 carboxy-terminal residues of prostatropin was derived by Edman degradation of overlapping peptides primarily generated by cleavage at lysyl and glutamyl residues. Analysis of the amino-terminal tetradecapeptide by fast atom bombardment mass spectrometry identified the blocking group as an acetyl moiety, and tandem mass spectrometry provided the sequence of the first 12 residues. Prostatropin residues 15–154 contain the sequence of bovine brain polypeptides recently described as acidic fibroblast growth factor and class I heparin-binding growth factor. The sequence of the first 25 residues of prostatropin is acetyl-Ala-(Gly, Glu)-Glu-Thr-Thr-Thr-Phe-Thr-Ala-Leu-Thr-Glu-Lys-Phe-Asn-Leu-Pro-Leu-Gly-Asn-Tyr-Lys-Lys-Pro. Reduced and carboxymethylated prostatropin exhibits mitogenic activity, suggesting that disulfide bonds among cysteine residues 30, 61, and 97 are not functionally essential. These results demonstrate by rigorous structural analysis that the brain-derived polypeptide previously described only as a mesenchymal and neuro-

1002319302

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

and acetylcholine were preserved when aortas were homogenized in buffer containing 3 mg/ml charcoal. Thus, most of the cyclic GMP-kinase activation occurred in the intact tissue and not because of endogenous cyclic nucleotides present during homogenization or assay. The increases in the activity ratio to sodium nitroprusside and acetylcholine correlate with increases in cyclic GMP concentration and with smooth muscle relaxation. It is concluded that cyclic GMP-kinase in rat aorta is activated by acetylcholine in an endothelium-dependent manner and by sodium nitroprusside in an endothelium-independent manner. These data are consistent with the hypothesis that cyclic GMP mediates relaxation of vascular smooth muscle to acetylcholine and sodium nitroprusside by activating cyclic GMP-kinase and consequent protein phosphorylation. The data further illustrate the importance of endothelial cells in vascular responses to acetylcholine.

Fiscus, R. R., Rapoport, R. M., and *Murad, F.*

Journal of Cyclic Nucleotide and Protein Phosphorylation Research 9(6):415-425, 1983-84.

Other support: National Institutes of Health, Veterans Administration and the American Heart Association, California Affiliate.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

EFFECTS OF THE D AND L STEREOISOMERS OF ISOIDIDE DINITRATE ON RELAXATION AND CYCLIC GMP ACCUMALATION IN RAT AORTA AND COMPARISON TO GLYCERYL TRINITRATE

The effects of the D- and L-stereoisomers of isoidide dinitrate on relaxation and cyclic GMP accumulation were compared in isolated rat aorta. Although both isomers were equally efficacious as vasorelaxants, the D-isomer was approximately ten times more potent that the L-isomer and about one-tenth the potency of glyceryl trinitrate. The median effective concentration of the D- and L-isomers for relaxation was 0.45 ± 0.06 μM and 6.7 ± 0.7 μM, respectively. The time-course of relaxation and elevation of cyclic GMP were closely correlated; the maximal relaxation and cyclic GMP accumulation occurred at 2 min. Consistent with the potency difference for relaxation, glyceryl trinitrate was ten times more potent than D-isoidide dinitrate, which was ten times more potent than the L-isomer with regard to the elevation of cyclic GMP levels. It is concluded that stereospecificity plays a role in organic nitrate-induced elevation of cyclic GMP and vasodilation.

Bennett, B. M., Hayward, L. D., and *Murad, F.*

Journal of Applied Cardiology 1:203-209, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA; and the Department of Chemistry, University of British Columbia, Vancouver, Canada.

1002312306

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

Tannenbaum, J. *et al.* (*Godman, G. C.*)

Journal of Cell Science **84**:253-262, 1986.

Other support: National Science Foundation, National Institutes of Health and the Muscular Dystrophy Foundation.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

CYTOCHALASIN D-INDUCED INCREASE IN ACTIN SYNTHESIS AND CONTENT IN A VARIETY OF CELL TYPES

Treatment of a variety of mesenchymal cells (normal and transformed rat fibroblasts, bovine aortic endothelial cells, rabbit smooth muscle cells), exhibiting different cytoskeletal organizations and derived from several species, with doses of cytochalasin D (CD, 2-6 μM for 20 h) sufficient to induce cytoskeletal rearrangement and altered cellular morphology results in an increase in the relative content and rate of synthesis of actin. These data extend our previous findings for HEp-2 cells to other cell types and provide further evidence for our hypothesis that the CD-induced cytoskeletal reorganization triggers stimulation of actin synthesis and the resulting increase in actin content.

Brett, J. G. and Tannenbaum, J. (*Godman, G. C.*)

Cell Biology International Reports **9**(8):723-730, August 1985.

Other support: National Science Foundation and National Institutes of Health.

From the Department of Pathology, Columbia University College of Physicians & Surgeons, New York.

EVIDENCE FOR REGULATION OF ACTIN SYNTHESIS IN CYTOCHALASIN D-TREATED HEp-2 CELLS

In HEp-2 cells treated with 0.2 or 2.0 μM cytochalasin D (CD), the relative rate of actin synthesis increased for about 12 h and then reached a plateau; this increase was suppressed by actinomycin D (AD). When CD was washed from cells which had been treated for 20 h, the elevated rate of actin synthesis declined to the control value within ca 4 h, as the actin-containing cytoskeletal components rearranged by CD recovered their normal morphology. Subsequently, actin synthesis was depressed below control values for a prolonged period; during recovery from 2 h treatment with CD, this depression was of much shorter duration. Re-addition of CD to cells after a 3 h recovery period again induced the cytoskeletal alterations characteristic of CD treatment but did not reverse the prior decline in the rate of actin synthesis. In HEp-2 cells treated with cycloheximide during exposure to CD for 20 h, the relative rate of actin synthesis measured after removal of cycloheximide was twofold higher than with CD alone and such cells exhibited a twofold slower decline in the rate of actin synthesis during recovery from CD in the continued presence of cycloheximide. These effects of cycloheximide, which resemble observations on "super-induction," suggest that actin synthesis in CD-treated and recovering HEp-2 cells may be regulated by a repressor

 1002319294

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

not normally present in these cells. Thus, synthesis of both muscle and non-muscle actins can be increased by CD, but enhancement of actin synthesis results from increases in the isoactins usually present, rather than induction of new isotypes. Comparison of CD-treated (fused) myotube cultures with (unfused) myoblast cultures indicated that β- and γ-actin synthesis was similarly enhanced in both cultures, but that α-actin synthesis was stimulated to a greater extent in the myoblast cultures. Desmin synthesis was also stimulated in the myoblasts but not the myotubes, suggesting that the effect of CD on synthesis of these developmentally regulated cytoskeletal proteins (α-actin, desmin) might be modulated by fusion or the state of differentiation of the muscle cell.

Tannenbaum, J. and Miranda, A. F. (*Godman, G. C.*)

Journal of Cell Science **84**:253-262, 1986.

Other support: National Science Foundation, National Institutes of Health and the Muscular Dystrophy Association.

From the Department of Pathology, College of Physicians & Surgeons of Columbia University, New York.

PHENOTYPIC AND KARYOTYPIC TRANSITIONS IN THE SPONTANEOUS TRANSFORMATION OF A RAT CELL LINE

After 20–50 transfers, a rat myofibroblast line, Hmf-n, "spontaneously" transforms to an established (immortalized) line of smaller, rapidly cycling fibroblastoid cells (tHmf-f). From these 1° transformants, colonies of larger, slower growing anchorage-independent (tHmf-e) cells of epithelioid phenotype emerge. Both transformants grow in low serum and low calcium media, but the tHmf-f cells are highly tumorigenic in nude mice, have diminished substrate adhesivity, and limited anchorage independence, whereas tHmf-e are less tumorigenic, firmly substrate adherent, and markedly anchorage independent. Most tHmf-f are trisomic; most tHmf-e transformants are hypodiploid, a third are tetraploid, and all have chromosomal abnormalities, but no trisomy. Hmf-n cells have polar stress fiber arrays terminating in vinculin adhesion plaques, colinear extracellular fibronectin matrices, and linear non-coincident deposits of fodrin. Microtubules (mt) and vimentin-intermediate filaments (IF) parallel the actin cables. Stress fibers of the tHmf-f are moderately reduced, their vinculin adhesion plaques and fibronectin matrices intact; fodrin is diffuse. Mts and IFs are normal and axial. Most epithelioid tHmf-e have no stress fibers, adhesion plaques, or extracellular fibronectin; instead, dense actin microfilament meshworks are attached to plasma membrane, as is fodrin. Mt and IF are radial. Both transformed phenotypes are stable over >300 continuous passages. The differentiation-inducing agents DMSO, cyclic AMP, 5-azacytidine, and mezerein, were ineffective in normalizing shape or cytoskeleton of transformed Hmf, and butyrate was selectively toxic to 50% of tHmf-e. But hydrocortisone induced striking polarization and increase in number and alignment of stress fibers of both tHmf-f and tHmf-e. Growth, anchorage, cytoskeletal arrangements, and tumorigenic potential are not closely correlated in these stable, spontaneously transformed lines of distinct pheno- and karyotype originating from the same normal parental cell, suggesting independent acquisition of properties associated with transformation.

1002319291

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

addition of cyanide. Incubation of nitroprusside with cyanide prior to addition to aortic strips did not alter the relaxant effect of nitroprusside. Sodium azide-, hydroxylamine-, N-methyl-N'-nitro-N-nitrosoguanide-, nitroglycerin- and acetylcholine-induced relaxations and increased levels of cyclic GMP were also inhibited by cyanide. Relaxations induced by nitric oxide were also inhibited by cyanide, although the relaxation with the low concentration of nitric oxide employed was not accompanied by detectable increases in cyclic GMP. Relaxation to 8-bromo-cyclic GMP was essentially unaltered by cyanide; however, isoproterenol-induced relaxation was inhibited. Guanylate cyclase in soluble and particulate fractions of aorta homogenates was activated by nitroprusside and the activation was prevented by cyanide. The present results suggest that cyanide inhibits nitrovasodilator-induced relaxation through inhibition of guanylate cyclase activation; however, cyanide may also have nonspecific effects which inhibit relaxation.

Rapoport, R. M. and *Murad, F.*

European Journal of Pharmacology **104**:61-70, 1984.

Other support: National Institutes of Health, Veterans Administration, and a National Research Service Award.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

SPECIFIC ATRIAL NATRIURETIC FACTOR RECEPTORS MEDIATE INCREASED CYCLIC GMP ACCUMULATION IN CULTURED BOVINE AORTIC ENDOTHELIAL AND SMOOTH MUSCLE CELLS

ANF is a newly discovered group of small peptides that exhibit potent vasodilatory activity.[1-5] In order to acquire a better understanding of the mechanism of action of ANF on vascular tissue, we used ^{125}I-ANF to identify receptors on cultured bovine aortic endothelial and smooth muscle cells. Scatchard analysis indicated that BASM cells contain a single class of high affinity binding sites with a K_D of 0.9 nM. BASM cells had 111,000 sites/cell. Similar results were recently reported by other investigators using rat[3] and bovine[4] aortic smooth muscle cells. We also have found that bovine aortic endothelial cells contain higher affinity (K_D = 0.1 nM) binding sites for ^{125}I-ANF (8–33), but a fewer number of receptors (16,000 sites/cell).[2] Five different atrial natriuretic peptides inhibited ^{125}I-ANF binding and stimulated cyclic GMP accumulation with the same order of potency when BASM or endothelial cells were examined. However, atriopeptin I was more effective at inhibiting ^{125}I-ANF binding than increasing cyclic GMP. While the K_i for atriopeptin I was about 6-fold higher than the K_i for ANF (8–33), it took at least a 100 times higher concentration for atriopeptin I to elicit the same magnitude of cyclic GMP stimulation in BASM cells. The disparity between the ability of atriopeptin I to inhibit ^{125}I-ANF binding and stimulate cyclic GMP was even more marked in endothelial cells.[2] In these cells the K_i for antriopeptin I to inhibit ^{125}I-ANF binding was 6-fold higher than the K_i for ANF (8–33), while the EC_{50} for atriopeptin I to increase cyclic GMP levels was at least 1500-fold greater. These results demonstrate that atriopeptin I is able to bind to ANF receptors effectively in both endothelial and smooth muscle cells, but is a weak stimulator of cyclic GMP accumulation.

Leitman, D. C., Waldman, S. A., Rapoport, R. M., and *Murad, F.*

1002313304

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

DESENSITIZATION TO NITROGLYCERIN IN VASCULAR SMOOTH MUSCLE FROM RAT AND HUMAN

Cyanylate cyclase in high speed supernatant fractions obtained from rat thoracic aorta or human coronary arteries pretreated with nitroglycerin exhibited a marked desensitization to activation by nitroglycerin, nitroprusside, and nitric oxide. However, activation of soluble guanylate cyclase by arachidonic acid was unaffected by pretreatment of vessels with nitroglycerin. Furthermore, activation of soluble guanylate cyclase by protoporphyrin IX was increased 4-fold when vessels were pretreated with nitroglycerin. Soluble guanylate cyclase partially purified from nitroglycerin-pretreated rat thoracic aorta by immunoprecipitation with a specific monoclonal antibody exhibited persistent desensitization to nitrate-induced activation. These date suggest that nitroglycerin-induced desensitization of guanylate cyclase to activation by nitrovasodilators represents a stable alteration of the enzyme. In contrast, activation by protoporphyrin IX of guanylate cyclase immunoprecipitated from nitroglycerin-pretreated or control vessels was not significantly different. This suggests that the mechanism of protoporphyrin activation of guanylate cyclase is different than the mechanism with nitrovasodilators. Activation of particulate guanylate cyclase by Lubrol-PX, hemin, or atrial natriuretic factor was not significantly different with enzyme prepared from nitroglycerin-pretreated or control vessels from rat and human. Thus, nitroglycerin-induced desensitization or rat thoracic aorta or human coronary artery results in a relatively stable molecular alteration of soluble guanylate cyclase such that the enzyme is specifically less sensitive to activation by nitrovasodilators, whereas the effects of other activators of the enzyme are either unchanged or increased.

Waldman, S. A., Rapoport, R. M., Ginsburg, R, *Murad, F.*

Biochemical Pharmacology **35**(20):3525-3531, 1986

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

IDENTIFICATION OF MULTIPLE BINDING SITES FOR ATRIAL NATRIURETIC FACTOR BY AFFINITY CROSS-LINKING IN CULTURED ENDOTHELIAL CELLS

In a previous study, we found that atriopeptin I was much weaker (EC_{50} > 500 nM) than atrial natriuretic factor (ANF-(8–33)) (EC_{50} = 0.3 nM) at increasing cyclic GMP in cultured endothelial cells. In this study, we used the cross-linking reagent disuccinimidyl suberate to investigate whether the differences in activity were due to the presence of multiple ANF receptors. When 98% of the ANF-binding sites on endothelial cells were occupied by tyrosine-atriopeptin I after cross-linking, there was no difference in the concentration-response curve to ANF-(8–33) with regard to cyclic GMP accumulation. In contrast, when 96% of the binding sites were occupied by cross-linked ANF-(8–33), a 60% decrease in the maximal cyclic GMP response was observed after the readdition of ANF-(8–33). These results suggest that ANF-(8–33) is binding to an additional site that atriopeptin I does not effectively bind. Affinity cross-linking of ^{125}I-ANF to intact endothelial cells resulted in the labeling of two sites of M_r ~66,000 and ~130,000. Approximately 94% of the ^{125}I-ANF binding sites had an M_r ~66,000. Labeling of this site was inhibited by both tyrosine-atriopeptin I (K_i = 0.9 nM) and

1002319312

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

placement at pH = 7.0. All three sets of experiments, (*i.e.*, free-exchange of bound $^{152}Eu^{3+}$, $^{151}Eu^{3+}$-induced displacement of bound $^{152}Eu^{3+}$ and ethidium ion-induced displacement of bound $^{152}Eu^{3+}$) indicate that the $^{152}Eu^{3+}$ ion can serve as a useful probe of metal ion and drug binding sites in nucleic acid polymers and constitutes a particularly sensitive probe at pH = 6.0.

Rosenthal, L. S., and *Nelson, D. J.*

Inorganica Chimica Acta **125**: 89-95, 1986.

Other support: National Science Foundation.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

FURTHER CHARACTERIZATION OF THE PLATINUM-REACTIVE COMPONENT OF THE α_2-MACROGLOBULIN-RECEPTOR RECOGNITION SITE

α_2-Macroglobulin (α_2M)–methylamine that had been allowed to react with *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) bound with greatly reduced affinity to specific α_2M receptors, as determined by macrophage binding studies *in vivo* and plasma-clearance experiments *in vivo*. Subsequent reaction with diethyl dithiocarbamate completely restored receptor recognition function. The optimal effect was obtained when the diethyl dithiocarbamate concentration was twice the total platinum concentration. α_2M–methylamine that was allowed to react with H_2O_2 competed less effectively for specific cell-surface binding sites, as demonstrated by studies both *in vivo* and *in vitro*. The apparent dissociation constant was increased nearly 7-fold by a 15 min exposure to H_2O_2. α_2M–methylamine was affected significantly less by the H_2O_2 exposure after pretreatment with *cis*-DDP. Amino acid analysis indicated that H_2O_2 treatment of α_2M modified 19 of the 25 methionine residues per α_2M subunit. Pretreatment with *cis*-DDP protected two to four of these methionine residues. The only other residue altered by H_2O_2 treatment of α_2M was histidine. A net decrease of two histidine residues per subunit was observed, but *cis*-DDP pretreatment did not alter this result. In order to rule out the slight possibility that histidine modification might account for the observed H_2O_2-induced loss in receptor recognition, diethyl pyrocarbonate was employed as a histidine-modifying reagent. This treatment modified 53 histidine residues in both native and fast-form α_2M. Fast-form α_2M was still recognized by the α_2M receptor, as determined by studies both *in vivo* and *in vitro*; however, a fraction of the modified protein now cleared via the acyl-low-density-lipoprotein receptor as well. Reaction of diethyl pyrocarbonate-treated α_2M with hydroxylamine reversed derivatization of 43 of the 53 histidine residues. Moreover, this treatment also resulted in an α_2M fast-form preparation that was recognized only by the α_2M receptor. It is concluded that *cis*-DDP and H_2O_2 modify a critical methionine residue in the primary sequence of the α_2M-receptor recognition site.

Pizzo, S. V. et al.

Biochemical Journal **238**:217-225, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology and Biochemistry, Duke University Medical Center, Durham, NC.

1002319315

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

IMAGING AT LOW LIGHT LEVEL IN FLUORESCENCE MICROSCOPY

Quantitative fluroescence microscopy is a powerful tool for studying the spatial and temporal dynamics of macromolecules, molecules and ions in living cells for at least five important reasons: (1) Sensitivity—with existing technology, as few as ca. 50 fluorescent molecules can be detected as a cluster on surfaces or inside cells. (2) Specificity—single classes of molecules can be studied even in a mixture of many other molecules by selecting the proper fluorescent probes and filter combinations. (3) Spectroscopy—fluorescence spectroscopic measurements such as fluorescence anisotropy and resonance energy transfer can yield molecular information about the molecules or the immediate environment. (4) Spatial resolution—the use of the fluorescence microscope permits the analysis of fluorescence parameters at the limit of resolution of the light microscope. Therefore, two- and three-dimensional maps of molecular distributions, as well as molecular activity, are possible in living cells. (5) Temporal resolution—fluorescence techniques are also valuable since they can detect and quantify those events which occur with a rate on the order of about 10^{3} sec^{-1} or slower.

The authors discuss some of the parameters that must be considered when a low-light-level video microscope system is used for quantitative imaging of fluorescent molecules. The parameters discussed are fundamental both for characterizing the limits of capability of the system as well as for providing the necessary data for partially rectifying some of these limitations by using image processing techniques.

Bright, G. R., and *Taylor, D. L.*

In: *Applications of Fluorescence in the Biomedical Sciences*, Alan R. Liss, Inc., 1986, Chap. 12, pp. 257-288.

Other support: National Institutes of Health and the Leukemia Society.

From the Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

FC-RECEPTOR-MEDIATED PHAGOCYTOSIS OCCURS IN MACROPHAGES WITHOUT AN INCREASE IN AVERAGE $[Ca^{++}]_i$

The calcium ion has been implicated as a cytosolic signal or regulator in phagocytosis. Using the Ca^{++}-sensitive photoprotein aequorin we have measured intracellular free Ca^{++} ion concentration ($[Ca^{++}]_i$) in thioglycolate-elicited mouse peritoneal macrophages during phagocytosis and IgG-induced spreading. Macrophages plated on glass were loaded with aequorin and $[Ca^{++}]_i$ was then measured from cell populations, both as previously described (McNeil, P. L., and D. L. Taylor, 1985, *Cell Calcium*, 6:83–92). Aequorin indicated a resting $[Ca^{++}]_i$ in adherent macrophages of 84 nM and was responsive to changes in $[Ca^{++}]_i$ induced by the addition of Mg-ATP (0.1 mM) or serum to medium. However, during the 15 min required for phagocytosis of seven or eight IgG-coated erythrocytes per macrophage loaded with aequorin, we measured no change in $[Ca^{++}]_i$. Similarly, the ligation of Fc-receptors that occurs when macrophages spread on immune complex-coated coverslips did not change macrophage $[Ca^{++}]_i$. In contrast, a rise in $[Ca^{++}]_i$ of macrophages was measured during phagocytosis occurring in a serum-free saline of pH 7.85, and as a consequence of incubation

100[illegible]9322

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

ably ANF binding occurs at the external membrane surface and cyclic GMP synthesis at the internal membrane surface of this transmembrane glycoprotein.

Kuno, T., Andresen, J. W., Kamisaka, Y., Waldman, S. A., Chang, L. Y., Saheki, S., Leitman, D. C., Nakane, M., and *Murad, F.*

The Journal of Biological Chemistry **261**(13):5817-5823, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

FORSKOLIN, PHOSPHODIESTERASE INHIBITORS, AND CYCLIC AMP ANALOGS INHIBIT PROLIFERATION OF CULTURED BOVINE AORTIC ENDOTHELIAL CELLS

The role of cyclic AMP on endothelial cell proliferation was investigated, since these cells can be exposed to high concentrations of physiological and pharmacological agents that alter cyclic AMP metabolism. Cloned bovine aortic endothelial cells were plated at 25,000 cells/35mm dish and grown for 5 days in the presence of phosphodiesterase (PDE) inhibitors, forskolin, or cyclic AMP analogs. The PDE inhibitors dipyridamole, ZK 62 711, isobutylmethylxanthine (IBMX) and theophylline inhibited cell growth in a concentration-dependent manner. Dipyridamole produced a 30% and a 50% inhibition at 5μM and 12.5μM, while higher concentrations were cytotoxic. At its therapeutic plasma concentration range (50–100μM) theophylline inhibited cell proliferation by 15–25%, while IBMX and the highly specific cyclic AMP phosphodiesterase inhibitor, ZK 62 711 inhibited growth by 60–80% and 40–50%, respectively. Forskolin (5μM) increased cyclic AMP levels and cyclic AMP-kinase activity ratios by 2.5-fold and 2-fold. In the absence of PDE inhibitors forskolin produced a 20% growth inhibition at 0.5μM and a 60% inhibition at 10μM. The forskolin dose-response curve was not altered by theophylline, but was shifted to the left by approximately 10-fold with dipyridamole and ZK 62 711 and 5-fold with IBMX. Forskolin (5μM), by itself produced a 1.8-fold increase in cyclic AMP. In the presence of 5μM theophylline, dipyridamole, IBMX, and ZK 62 711, cyclic AMP was increased by forskolin 2.0, 2.6, 3.5, and 6.6-fold, respectively. 8-Bromo cyclic AMP and dibutyryl cyclic AMP produced a 55% and 60% growth inhibition at 100μM. The cyclic GMP analogs were ineffective inhibitors of growth (15–30%). Our results demonstrate that cyclic AMP analogs and pharmacological agents that elevate intracellular cyclic AMP levels inhibit cell growth and suggest that cyclic AMP may be an important endogenous regulator of endothelial cell proliferation.

Leitman, D. C., Fiscus, R. R. and *Murad, F.*

Journal of Cellular Physiology **127**:237-243, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

1002319311

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

displays cooperative kinetics toward NADPH (Hill coefficient (n_H) = 1.97 ± 0.07), the enzyme from CM microsomes does not (n_H) = 1.04 ± 0.07). Similarly to HMG-CoA reductase from CM microsomes, the freeze-thaw solubilized enzyme ("SOL") displays no cooperativity toward NADPH and its K_m for this substrate is 34 μM.

At 4.5 mM GSH, HMG-CoA reductase from X, CM, and SOL preparations has a similar K_m value for [DL]-HMG-CoA, ranging between 13–16 μM, while reductase from N microsomes had a higher K_m value (42 μM) for this substrate. No cooperativity towards HMG-CoA was observed in any of the tested enzyme preparations.

Immunoblotting analyses of the different preparations demonstrated that the observed altered kinetics of HMG-CoA reductase in the microsomes are not due to preferential proteolytic cleavage of the native 97–100 kDa subunit of the enzyme to the noncooperative 50–55 kDa species. Moreover, it was found that the ratio enzymatic activity/immunoreactivity of the reductase increased in the order N<X<CM≅SOL, indicating that the activity per reductase molecule increases with the induction of the enzyme. These results are compatible with a model suggesting that dietary induction of hepatic HMG-CoA reductase may change the state of functional aggregation of its subunits.

Roitelman, J., and *Shechter, I.*

The Journal of Biological Chemistry **261**(11):5061-5066, 1986.

From the Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

STRUCTURE AND MOBILITY OF ACTIN FILAMENTS AS MEASURED BY QUASIELASTIC LIGHT SCATTERING, VISCOMETRY, AND ELECTRON MICROSCOPY

Actin filaments of different lengths were prepared by polymerizing actin in the presence of various concentrations of gelsolin, a protein which accelerates actin polymerization by stabilizing nuclei from which filaments grow and which binds to their fast growing ends. The lengths of the actin filaments following polymerization were measured by electron microscopy and showed that the number-average filament length agreed with the predicted length if each gelsolin molecule acted as a seed for the growth of an actin filament. The distribution of lengths was independent of the actin: gelsolin ratio and was similar to that of actin filaments polymerized in the absence of gelsolin ($L_w/L_n = 1.8$). The mobility of these filaments in solution was studied by quasielastic light scattering and by viscometry. The translational diffusion constant determined by quasielastic light scattering was in agreement with the infinite dilution values calculated from the dimensions and the distributions of lengths determined by electron microscopy for relatively short filament lengths. Under conditions where overlap of the rotational domains of the filaments would be expected to occur, the measured diffusion rates deviated from their predicted dilute solution values and the solution viscosity increased abruptly. The dependence of the diffusion constant and the solution viscosity on the length of the actin filaments can be explained in terms of a theory that describes the restraints on diffusion of independent rigid rods in semi-dilute solution. The results suggest that the rheology of actin filaments can be accounted for by steric restraints. The length of cytoplasmic actin filaments in some cell types is such that these steric constraints are significant and could produce large changes in physical properties with small changes in filament length.

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

derived macrophages and this was not affected by either phorbol or A23187. Thus these two antigens, despite coordinate expression in response to IFN-γ in normal mouse strains, are clearly regulated independently. These results suggest that IFN-γ generates at least two independent molecular events in macrophages which ultimately modulate the expression of cell surface proteins important to the performance of activated functions.

Strassmann, G., Somers, S. D., Springer, T. A., *Adams, D. O.* and Hamilton, T. A.

Cellular Immunology **97**:110-120, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC, and the Laboratory for Membrane Immunochemistry, Dana Farber Cancer Center, Harvard University Medical Center, Boston.

LPS INDUCES ALTERED PHOSPHATE LABELING OF PROTEINS IN MURINE PERITONEAL MACROPHAGES

Covalent modification of proteins via phosphorylation is a well-documented mechanism whereby intracellular events are controlled by external stimuli. Treatment of thioglycollate-elicited, C57Bl/6 murine peritoneal macrophages with nanogram quantities of bacterial lipopolysaccharide (LPS) consistently results in altered ^{32}Pi labeling of a specific set of proteins (*e.g.*, proteins of 67, 37, 33, and 28 kD), as measured by autoradiography after SDS-polyacrylamide gel electrophoresis. Induction of this pattern of phosphorylation is duplicated by the lipid A moiety of LPS. The LPS-stimulated changes in phosphate labeling are both dose- and time-dependent. The phosphorylation pattern induced in macrophages by the tumor promoter phorbol myristic acetate, one of various pharmacologic agents tested, shows similarity to the pattern induced by LPS. Analysis of pp 28 and pp 37 from both LPS- and PMA-treated macrophages by limited proteolysis demonstrates that these phosphoproteins are structurally related and that the sites of phosphorylation are similar for both treatment conditions. Macrophages from the genetically LPS-unresponsive C3H/HeJ strain show no alteration in their pattern of phosphorylation after treatment with LPS. Control macrophages, from C3H/HeN mice, respond to LPS in a fashion identical to that seen in C57Bl/6 macrophages. Pretreatment of macrophages with IFN-γ potentiates the effect of LPS (*i.e.*, yields a level of altered phosphate labeling greater than observed with LPS or PMA alone). Together, the data indicate that LPS causes altered phosphate labeling of a defined set of proteins, and that the circumstances of this response are consistent with a possible role in coupling LPS-initiated signals to the induction of functional competence in macrophages.

Weiel, J. E., Hamilton, T. A. and *Adams, D. O.*

The Journal of Immunology **136**(8):3012-3018, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

1002319329

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

with quin2/AM. We estimate that had a change in $[Ca^{++}]$ occurred during phagocytosis, aequorin would have detected a rise from 0.1 to 1.0 μM taking place in as little as 2% of the macrophage's cytoplasmic volume. We therefore suggest that either Ca^{++} is not involved as a cytoplasmic signal for phagocytosis or that increases in $[Ca^{++}]$ during phagocytosis are confined to such small regions of cytoplasm as to be below the limits of detection by our cellular averaging method. Our data emphasizes, moreover, the need for well-defined, nonperturbing conditions in such measurements of $[Ca^{++}]$.

McNeil, P. L., Swanson, J. A., Wright, S. D., and *Taylor, D. L.*

The Journal of Cell Biology **102**:1586-1592, May 1986.

Other support: U. S. Public Health Service and the American Cancer Society.

From the Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

A TRANSIENT RISE IN CYTOSOLIC CALCIUM FOLLOWS STIMULATION OF QUIESCENT CELLS WITH GROWTH FACTORS AND IS INHIBITABLE WITH PHORBOL MYRISTATE ACETATE

We have used aequorin as an indicator for the intracellular free calcium ion concentration ($[Ca^{++}]_i$) of Swiss 3T3 fibroblasts. Estimated $[Ca^{++}]_i$ of serum-deprived, subconfluent fibroblasts was 89 (±20) nM, almost twofold higher than that of subconfluent cells growing in serum, whose $[Ca^{++}]_i$ was 50 (±19) nM. Serum, partially purified platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) stimulated DNA synthesis by the serum-deprived cells, whereas epidermal growth factor (EGF) did not. Serum immediately and transiently elevated the $[Ca^{++}]_i$ of serum-deprived cells, which reached a maximal value of 5.3 μM at 18 s poststimulation but returned to near prestimulatory levels within 3 min. Moreover, no further changes in $[Ca^{++}]_i$ were observed during 12 subsequent h of continuous recording. PDGF produced a peak rise in $[Ca^{++}]_i$ to ~1.4 μM at 115 s after stimulation, and FGF to ~1.2 μM at 135 s after stimulation. EGF caused no change in $[Ca^{++}]_i$. The primary source of calcium for these transients was intracellular, since the magnitude of the serum-induced rise in $[Ca^{++}]_i$ was reduced by only 30% in the absence of exogenous calcium. Phorbol 12-myristate 13-acetate (PMA) had no effect on resting $[Ca^{++}]_i$. When, however, quiescent cells were treated for 30 min with 100 nM PMA, serum-induced rises in $[Ca^{++}]_i$ were reduced by sevenfold. PMA did not inhibit growth factor-induced DNA synthesis and was by itself partially mitogenic. We suggest that if calcium is involved as a cytoplasmic signal for mitogenic activation of quiescent fibroblasts, its action is early, transient, and can be partially substituted for by PMA. Activated protein kinase C may regulate growth factor-induced increases in $[Ca^{++}]_i$.

McNeil, P. L., McKenna, M. P. and *Taylor, D. L.*

The Journal of Cell Biology **101**:372-379, August 1985.

Other support: National Institutes of Health.

Department of Biological Sciences, Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon, Pittsburgh.

1002319323

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

consecutive first-order processes, and the fraction of label associated with each rate can be determined. Studies of phosphodiesterase activation by calmodulin show Cd^{2+} and calmodulin to cause 80% of the maximum activation found when Ca^{2+} and calmodulin are used.

Buccigross, J. M., O'Donnell, C. L., and *Nelson, D. J.*

Biochemical Journal **235**:677-684, 1986.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

SOLVENT INTERACTIONS WITH *N,N*-DIALKYLNICOTINAMIDES AND THEIR EFFECTS ON ROTATIONAL BARRIERS

Carbon-13 nuclear magnetic resonance techniques were employed to examine the effects of solvent environment on rotational barriers in a series of molecules structurally-related to the analeptic, nikethamide: *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine. Total bandshape analysis was performed for the exchanging alkyl carbon resonances of these compounds as a function of temperature in four solvent systems: D_2O, CH_3OD, CH_3CH_2OD and $CDCl_3$. The rate constants for rotation about the amide bond obtained in this way were used to calculate free energy ($\Delta G\ddagger$), enthalpy ($\Delta H\ddagger$) and entropy ($\Delta S\ddagger$) of activation parameters for this process. Our results indicate that rotational barriers are less affected by the nature of the alkyl chain attached to the amide nitrogen than by the size and polarity of the solvent molecules. Interpretation of the thermodynamic parameters in light of both nikethamide analogue structure and solvent type has further clarified the manner in which hydrogen bonding interactions between solvent molecules and the carbonyl oxygen of these analogues stabilize transition state conformers.

Bean, J. W., *Nelson, D. J.*, and Wright, G. E.

Biochemical Pharmacology **35**(6):1011-1017, 1986.

Other support: National Science Foundation.

From the Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA.

$^{153}Eu^{3+}$ AS A PROBE OF METAL ION AND CATIONIC DRUG BINDING SITES ON NATIVE AND HEAT-DENATURED DNA

A flow-dialysis apparatus suitable for the study of high affinity metal ion binding sites in macro-molecules has been utilized to study $^{153}Eu^{3+}$ exchange processes, as a function of pH, in both "native" and "heat-denatured" DNA. "Free exchange" of $^{153}Eu^{3+}$ was found to occur at a significantly faster rate at pH = 7.0 than at pH = 6.0 for both forms of DNA; while non-radioactive $^{153}Eu^{3+}$-induced "displacement" of bound $^{153}Eu^{3+}$ occurred at a significantly faster rate at pH = 6.0 than at pH = 7.0 for both species of DNA. These results are consistent with a greater "entropic" driving force for metal ion:DNA complexation at the lower pH value. The effect of ethidium bromide on $^{153}Eu^{3+}$ exchange was also examined as a function of pH. The intercalating agent was found to accelerate $^{153}Eu^{3+}$ displacement at pH = 6.0 and decelerate dis-

1002319314

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

COMPARISON OF BINDING AND CYCLIC GMP ACCUMULATION BY ATRIAL NATRIURETIC PEPTIDES IN ENDOTHELIAL CELLS

Rat ^{125}I-labeled atrial natriuretic factor (ANF (8–33)) was used to identify ANF receptors on cultured bovine aortic endothelial cells. Specific binding of ^{125}I-ANF at 37°C to confluent endothelial cells was saturable and of high affinity. Scatchard analysis of the equilibrium binding data indicated that endothelial cells contain a single class of binding sites with a K_d of 0.1 ± 0.01 nM. This particular clone of endothelial cells had 16000 ± 1300 receptors per cell. The order of potency for competing with ^{125}I-ANF binding was human atrial natriuretic peptide (hANP) = atrial natriuretic factor (ANF (8–33)) > atriopeptin II > atriopeptin III > atriopeptin. The weakest competitor, atriopeptin I, had a K_i of 0.45 nM, which was only 6-fold higher than the K_i for hANP and ANF (8–33). ANF (8–33) and hANP in the presence of 0.5 mM isobutylmethylxanthine produced a 15–20-fold increase in cyclic GMP content at 10 pM and a maximal 500-fold elevation of cyclic GMP at 10 nM. The concentrations required to elicit a half-maximal increase in cyclic GMP for hANP, ANF (8–33), atriopeptin I, atriopeptin II and atriopeptin III were 0.30, 0.35, > 500, 4.0 and 5.0 nM, respectively. Although atriopeptin I acted as a partial agonist, it was unable to antagonize the effect of ANF (8–33) on cyclic GMP formation. These findings suggest that endothelial cells have multiple and functionally distinct ANF-binding sites.

Leitman, D. C. and *Murad, F.*

Biochimica et Biophysica Acta **885**:74–79, 1986.

Other support: National Insitutes of Health and the Veterans Administration.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CO-PURIFICATION OF AN ATRIAL NATRIURETIC FACTOR RECEPTOR AND PARTICULATE GUANYLATE CYCLASE FROM RAT LUNG

An atrial natriuretic factor (ANF) receptor from rat lung was solubilized with Lubrol-PX and purified by sequential chromatographic steps on GTP-agarose, DEAE-Sephacel, phenyl-agarose, and wheat germ agglutinin-agarose. The ANF receptor was enriched 19,000-fold. The purified receptor has a binding profile and properties that correspond to the affinity and specificity found in membranes and crude detergent extracts. Polyacrylamide gel electrophoresis of the purified preparation in the presence of sodium dodecyl sulfate and dithiothreitol showed the presence of one major protein band with a molecular mass of 120,000 daltons. When purified preparations were incubated with ^{125}I-ANF, then cross-linked with disuccinimidyl suberate, the 120,000-dalton protein was specifically radiolabeled. This high affinity binding site for ANF co-purified with particulate guanylate cyclase. Particulate guanylate cyclase was purified to a specific activity of 19 μmol cyclic GMP produced/min/mg of protein utilizing Mn-GTP as substrate. This represented a 15,000-fold purification compared to the initial lung membrane preparation with Lubrol-PX. Gel permeation high performance liquid chromatography and glycerol density gradient sedimentation studies of the purified preparation also resulted in co-migration of specific ANF binding and guanylate cyclase activities. The co-purification of these activities suggests that both ANF binding and guanylate cyclase activities reside in the same macromolecular complex. Presum-

1002319310

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

VI. *Immunology and Adaptive Mechanisms*

REGULATION OF THE Fc-RECEPTOR-MEDIATED RESPIRATORY BURST: TREATMENT OF PRIMED MURINE PERITONEAL MACROPHAGES WITH LIPOPOLYSACCHARIDE SELECTIVELY INHIBITS H_2O_2 SECRETION STIMULATED BY IMMUNE COMPLEXES

The effect of bacterial lipopolysaccharide on the Fc-receptor-mediated respiratory burst in murine peritoneal macrophages has been examined. After treatment overnight with small quantities of LPS, macrophages exhibited dramatic diminution of their capacity to generate and secrete H_2O_2 when triggered with immune complexes. The effect of LPS treatment was dependent on the state of macrophage functional activation; only cells that were primed or fully activated in vivo or were treated with interferon-γ in vitro were sensitive to this effect of LPS. The LPS-mediated loss of secretory function was both dose and time dependent and could be reproduced with the lipid A moiety of LPS. The effect was selective for H_2O_2 secretion triggered through the Fc receptor; the respiratory burst stimulated by phorbol diesters remained unaltered. Furthermore, LPS treatment did not alter either binding or ingestion of radiolabeled immune complexes in parallel with the change in H_2O_2 secretion, indicating that the suppressive effect was not due to compromised endocytic function. These results indicate that LPS treatment of primed macrophages regulates the function of Fc receptors and may uncouple receptor occupancy from generation and secretion of H_2O_2.

Johnston, P. A., *Adams, D. O.* and Hamilton, T. A.

The Journal of Immunology **135**(1):513-518, 1985.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

BIOCHEMICAL MODELS OF INTERFERON-γ-MEDIATED MACROPHAGE ACTIVATION: INDEPENDENT REGULATION OF LYMPHOCYTE FUNCTION ASSOCIATED ANTIGEN (LFA)-1 AND I-A ANTIGEN ON MURINE PERITONEAL MACROPHAGES

IFN-γ can induce the expression of both class II histocompatibility antigens (Ia) and the lymphocyte function associated (LFA)-1 antigen on murine peritoneal macrophages. We have examined the molecular changes which lead to altered expression of these two cell surface proteins to determine whether they are regulated by similar or independent mechanisms. While I-A antigen expression can be induced or enhanced by treatment of macrophages with either phorbol diesters and/or the Ca^{++} ionophore A23187, these agents had no effect upon expression of LFA-1 under similar conditions. Macrophages from the A/J strain mouse exhibit a deficiency in their sensitivity to IFN-γ which is seen in our studies as an inability of IFN-γ to elevate I-A antigen expression. However, expression of I-A could be modulated in these cells by treatment with either phorbol diesters or A23187. In contrast, IFN-γ could induce LFA-1 antigen on A/J

1002319328

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

detergents and chaotropes. Particulate guanylate cyclase and the ST receptor solubilized by detergent retained their abilities to produce cyclic GMP and bind ST, respectively. However, ST failed to activate particulate guanylate cyclase in detergent extracts. In contrast, guanylate cyclase resistant to solubilization remained functional and coupled to the ST receptor since enzyme activation by ST was unaffected by various extraction procedures. The possibility that the ST receptor and particulate guanylate cyclase were the same molecule was explored. ST binding and cyclic GMP production were separated by affinity chromatography on GTP-agarose. Similarly, guanylate cyclase migrated as a 300,000-dalton protein, while the ST receptor migrated as a 240,000-dalton protein on gel filtration chromatography. Also, thiol-reactive agents such as cystamine and *N*-ethylmaleimide inhibited guanylate cyclase activation by ST, with no effect on receptor binding of ST. These data suggest that guanylate cyclase and the ST receptor are independent proteins coupled by cytoskeletal components in membranes of intestinal mucosa.

Waldman, S. A., Kuno, T., Kamisaki, Y., Chang, L. Y., Gariepy, J., O'Hanley, P., Schoolnik, G., and *Murad, F.*

Infection and Immunity **51**(1):320-326, 1986.

Other support: National Institutes of Health and the Veterans Administration.

From the Departments of Medicine, Pharmacology, and Microbiology, Stanford University School of Medicine, Stanford, CA, and the Veterans Administration Medical Center, Palo Alto, CA.

CYCLIC GUANOSINE MONOPHOSPHATE AS A MEDIATOR OF VASODILATION

Although cyclic guanosine monophosphate (GMP) was first described in biological samples more than two decades ago, its role in some physiological processes has only become apparent in the past few years. This relatively slow development is probably attributable to the low concentrations of the nucleotide in tissues, the complex and insensitive methods available during the early studies, and the biases many investigators had regarding its possible functions. The latter was undoubtedly influenced by the many similarities of the cyclic GMP system with that of cyclic AMP and the attention cyclic AMP has received during this period. While analogies and similarities between these two cyclic nucleotide systems do exist, the cyclic GMP system presents more complexities due to the existence of several isoenzymes responsible for its synthesis. It is known that the conversion of guanosine triphosphate (GTP) to cyclic GMP is catalyzed by at least two isoenzyme forms of guanylate cyclase. The kinetic, physicochemical, and antigenic properties of the cytosolic and membrane-associated isoenzymes are quite different. The relative abundance of the soluble and particulate enzyme is variable in different tissues and species. While intestinal mucosa and retina possess predominately the particulate isoenzyme and platelets contain the soluble isoenzyme, most tissues such as vascular smooth muscle have both isoenzymes. Furthermore, the regulation of each of these isoenzymes is quite different. The soluble enzyme appears unique in that it can be activated by reactive free radicals such as nitric oxide,

1002313308

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

ANF-(8–33) $K_i = 0.9$ nM). Although 0.1 μM tyrosine-atriopeptin (AP I) inhibited labeling of the 66,000-dalton site to nearly the same degree as ANF-(8–33), it produced only a 4-fold increase in cyclic GMP compared to a 400-fold increase with ANF-(8–33). These results suggest that the 66,000-dalton site is not coupled to guanylate cyclase and cyclic GMP formation. Tyrosine-AP I ($K_i > 10$ nM) was much weaker at competing for the 130,000-dalton site than ANF-(8–33) ($K_i = 0.075$ nM). Because the EC_{50} for cyclic GMP stimulation for tyrosine-AP I (>100 nM) and ANF-(8–33) (0.4 nM) is closer to the K_i values for the 130,000-dalton protein, this site probably mediates the marked stimulation of cyclic GMP. Our results demonstrate that endothelial cells contain two binding sites for ANF-(8–33) and suggest that only the less abundant site ($M_r \sim 130,000$) is the receptor coupled to the activation of guanylate cyclase.

Leitman, D. C., Andresen, J. W., Kuno, T., and *Murad, F.*

The Journal of Biological Chemistry **261**(25):11650-11655, 1986.

Other support: National Institutes of Health.

From the Department of Medicine and Pharmacology, Stanford University School of Medicine, Stanford, CA.

EPR STUDIES SHOW THAT ALL LANTHANIDES DO NOT HAVE THE SAME ORDER OF BINDING TO CALMODULIN

Calmodulin, spin labeled at Tyr-99, has been titrated with the lanthanides La^{3+}, Nd^{3+}, Eu^{3+}, Tb^{3+}, Er^{3+} and Lu^{3+} as well as Ca^{2+} and Cd^{2+}. The titration was monitored by EPR and changes in mobility of the spin label, due to binding into the labeled site and protein conformational change, were observed. Comparison of these titration curves with theoretical binding curves for the various calmodulin-metal species, show that different lanthanides have different high affinity sites. Three basic categories were observed, with Lu^{3+} and Er^{3+} behaving like Ca^{2+}, Eu^{3+} and Tb^{3+} binding in the opposite order from Ca^{2+}, and La^{3+} and Nd^{3+} different from either Ca^{2+} or Tb^{3+}.

Buccigross, J. M. and *Nelson, D. J.*

Biochemical and Biophysical Research Communications **138**(3):1243-1249, 1986.

From the Department of Chemistry, Clark University, Worcester, MA.

A FLOW-DIALYSIS METHOD FOR OBTAINING RELATIVE MEASURES OF ASSOCIATION CONSTANTS IN CALMODULIN-METAL-ION SYSTEMS

A flow-dialysis apparatus suitable for the study of high-affinity metal-binding proteins has been utilized to study calmodulin–metal exchange as a measure of relative calmodulin–metal association constants. Calmodulin labelled with radioactive ^{153}Gd was dialysed against buffer containing various competing metal ions. The rate of label exchange was monitored by a γ-ray scintillation detector. Competing metals used were Ca^{2+} and Cd^{2+}, and the lanthanides Gd^{3+}, Eu^{3+}, La^{3+} and Lu^{3+}. All exchange processes were first-order, and two categories of metal were found: Ca^{2+} and Cd^{2+} in one, the lanthanides comprising the other. In addition calmodulin–metal complexes with radioactive ^{109}Cd and ^{45}Ca released the bound label without any competing metal being added to the buffer. The kinetics of this metal loss can be described by two

1002319313

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

human and bovine blood coagulation proteases, and human factor D by substituted isocoumarins and phthalides which contain masked acyl chloride or anhydride moieties, are reported. 3,4-Dichloroisocoumarin, the most potent inhibitor investigated here, inactivated all the serine proteases tested but did not inhibit papain, leucine aminopeptidase, or β-lactamase. 3,4-Dichloroisocoumarin was fairly selective toward HL elastase ($K_{inact}/[I]$ = 8920 M^{-1} s^{-1}); the inhibited enzyme was quite stable to reactivation (K_{react} = 2 × 10^{-5} s^{-1}), while enzymes inhibited by 3-acetoxyisocoumarin and 3,3-dichlorophthalide regained full activity upon standing. The rate of inactivation was decreased dramatically in the presence of reversible inhibitors or substrates, and ultraviolet spectral measurements indicate that the isocoumarin ring structure is lost upon inactivation. Chymotrypsin A_γ is totally inactivated by 1.2 equiv of 3-chloroisocoumarin or 3,4-dichloroisocoumarin, and approximately 1 equiv of protons is released upon inactivation. These results indicate that these compounds react with serine proteases to release a reactive acyl chloride moiety which can acylate another active site residue. These are the first mechanism-based inhibitors reported for many of the enzymes tested, and 3,4-dichloroisocoumarin should find wide applicability as a general serine protease inhibitor.

Harper, J. W., Hemmi, K., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:1831-1841, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

REACTION OF SERINE PROTEASES WITH SUBSTITUTED 3-ALKOXY-4-CHLOROISOCOUMARINS AND 3-ALKOXY-7-AMINO-4-CHLOROISOCOUMARINS: NEW REACTIVE MECHANISM-BASED INHIBITORS

The time-dependent inactivation of several serine proteases including human leukocyte elastase, cathepsin G, rat mast cell proteases I and II, and human skin chymase by a number of 3-alkoxy-4-chloroisocoumarins, 3-alkoxy-4-chloro-7-nitroisocoumarins, and 3-alkoxy-7-amino-4-chloroisocoumarins at pH 7.5 and the inactivation of several trypsin-like enzymes including human thrombin and factor XIIa by 7-amino-4-chloro-3-ethoxyisocoumarin and 4-chloro-3-ethoxyisocoumarin are reported. The 3-alkoxy substituent of the isocoumarin is likely interacting with the S_1 subsite of the enzyme since the most reactive inhibitor for a particular enzyme had a 3-substituent complementary to the enzyme's primary substrate specificity site (S_1). Inactivation of several enzymes including human leukocyte elastase by the 3-alkoxy-7-amino-4-chloroisocoumarins is irreversible, and less than 3% activity is regained upon extensive dialysis of the inactivated enzyme. Addition of hydroxylamine to enzymes inactivated by the 3-alkoxy-7-amino-4-chloroisocoumarins results in a slow ($t_{1/2}$ > 6.7 h) and incomplete (32–57%) regain in enzymatic activity at pH 7.5. Inactivation by the 3-alkoxy-4-chloroisocoumarins and 3-alkoxy-4-chloro-7-nitroisocoumarins on the other hand is transient, and full enzyme activity is regained rapidly either upon standing, after dialysis, or upon the addition of buffered hydroxylamine. The rate of inactivation by the substituted isocoumarins is decreased when substrates or reversible inhibitors are present in the incubation mixture, which indicates active site involvement. The inactivation rates are dependent upon the pH of the reaction mixture, the isocoumarin ring system is opened concurrently with inactivation, and the reaction of

1002319326

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

3-alkoxy-7-amino-4-chloroisocoumarins with porcine pancreatic elastase is shown to be stoichiometric. The results are consistent with a scheme where 3-alkoxy-7-amino-4-chloroisocoumarins react with the active site serine of a serine protease to give an acyl enzyme in which a reactive quinone imine methide can be released. Irreversible inactivation could then occur upon alkylation of an active site nucleophile (probably histidine-57) by the acyl quinone imine methide. The finding that hydroxylamine slowly catalyzes partial reactivation indicates that several inactivated enzyme species may exist. The 3-alkoxy-substituted 4-chloroisocoumarins and 4-chloro-7-nitroisocoumarins are simple acylating agents and do not give stable inactivated enzyme structures. Substituted isocoumarins are some of the most potent inactivators reported for many of the enzymes tested and may be quite useful as inhibitors of proteolysis both *in vivo* and *in vitro*.

Harper, J. W., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:7200-7213, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

VARIABILITY OF FUNCTIONAL CHARACTERISTICS OF MDCK CELLS

We measured several functional parameters of MDCK cells cultured as monolayers in order to more fully characterize their ion transport properties. Most of the present studies were completed with five groups (*A–E*) of MDCK cells studied from passage 62 to 78. Each group represents the same subline of MDCK cells after having been frozen, stored, and thawed at passage 62 or 64. The median transmonolayer resistances of the groups were 507, 149, 284, 72, and 126 $\Omega \cdot cm^2$. Addition of amphotericin B to the apical solution induced a ouabain-sensitive transepithelial current. The apical membrane voltage and fractional resistance exhibited a wide range of values in two of the groups studied, with mean values of -32 mV and 0.68 in *group B* and -40 mV and 0.78 in *group E*. Neither apical nor basolateral membrane displayed significant Na^+ conductance. K^+ conductance was present in the basolateral but not in the apical membrane. Acidification or alkalinization of the apical solution was dependent on the conditions used to study the cells. The 4,4'-diisothyocyano-2,2'-disulfonic stilbene inhibited acidification (or induced alkalinization), whereas increasing ambient HCO_3^- concentration induced alkalinization. The results of these studies indicate qualitatively similar behavior between five groups of MDCK cells but significant quantitative differences between the groups. Analysis of the variability of the measured parameters indicated that there were no differences as a function of passage number within a group. The factors responsible for functional differences between groups are not known but may be related to the cell storage process.

Husted, R. F., *Welsh, M. J.*, and Stokes, J. B.

American Journal of Physiology **250**:C214-C221, 1986.

Other support: National Heart, Lung and Blood Institute and the Cystic Fibrosis Foundation.

From the Laboratory of Epithelial Transport and Kidney Physiology, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City.

1002319327

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS ON CALCIUM AND CENTRILOBULAR NECROSIS IN THE LIVER OF RATS TREATED WITH HEPATOTOXIC AGENTS

Carbon tetrachloride, chloroform, dimethylnitrosamine, thioacetamide or acetaminophen was each administered to rats in a single hepatotoxic dose. Nifedipine, verapamil or chlorpromazine was administered in association with the hepatotoxic agents to determine if calcium channel blocking agents would prevent an increase in liver cell calcium associated with hepatotoxicity and to determine if these agents would protect against the development of centrilobular necrosis. Following a latent period different for each toxic agent, a 4- to 18-fold increase in liver cell calcium content had occurred by 24 hr. The calcium increase and the centrilobular necrosis (mean histologic score) were correlated. A relatively high calcium to necrosis ratio was obtained with dimethylnitrosamine, thioacetamide and acetaminophen. A lesser calcium to necrosis ratio was obtained with chloroform and carbon tetrachloride, the two toxic agents that destroyed the intracellular calcium sequestration activity of the liver endoplasmic reticulum. Nifedipine or chlorpromazine, administered prior to and 7 hr after the toxic agent, completely prevented the centrilobular necrosis caused by thioacetamide, carbon tetrachloride and acetaminophen; almost completely prevented necrosis with dimethylnitrosamine; and provided partial protection against chloroform toxicity. Two doses of verapamil provided partial protection against necrosis when carbon tetrachloride was the toxic agent and provided almost complete protection with dimethylnitrosamine. A reduction in liver cell calcium was associated with the protective action of the three calcium channel blocking agents. These findings are compared with earlier studies of the protective effects of calcium channel blocking agents in cardiac ischemia.

Landon, E. J., Naukam, R. J., and *Sastry, B. V. R.*

Biochemical Pharmacology **35**(4):697-705, 1986.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

ALTERED KINETIC PROPERTIES OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE FOLLOWING DIETARY MANIPULATIONS

The microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the rate-limiting step in the cholesterogenic pathway and was proposed to be composed *in situ* of 2 noncovalently linked subunits. In the present report, the activities and kinetic properties of HMG-CoA reductase in microsomes isolated from livers of rats fed on diets supplemented with either ground Amberlite XAD-2 ("X"), cholestyramine/mevinolin ("CM"), or unsupplemented normal rat chow ("N"), were compared.

The specific activities of HMG-CoA reductase in X and CM microsomes were, respectively, 5- and 83-fold higher than that of N microsomes. In NADPH-dependent kinetics of HMG-CoA reductase activated with 4.5 mM GSH, the concentration of NADPH required for half-maximal velocity ($S_{0.5}$) was 209 ± 23, 76 ± 23, and 40 ± 4 μM for the N, X, and CM microsomes, respectively. While reductase from X microsomes

100231931S

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

Janmey, P. A., Peetermans, J., Zaner, K. S. and *Stossel, T. P.*

The Journal of Biological Chemistry **261**(18):8357-8362, 1986.

Other support: U. S. Public Health Service and the Richard Saltonstall Endowment.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

THE ARCHITECTURE OF ACTIN FILAMENTS AND THE ULTRASTRUCTURAL LOCATION OF ACTIN-BINDING PROTEIN IN THE PERIPHERY OF LUNG MACROPHAGES

A highly branched filament network is the principal structure in the periphery of detergent-extract cytoskeletons of macrophages that have been spread on a surface and either freeze or critical point dried, and then rotary shadowed with platinum-carbon. This array of filaments completely fills lamellae extended from the cell and bifurcates to form 0.2-0.5 μm thick layers on the top and bottom of the cell body. Reaction of the macrophage cytoskeletons with anti-actin IgG and with anti-IgG bound to colloidal gold produces dense staining of these filaments, and incubation with myosin subfragment 1 uniformly decorates these filaments, identifying them as actin. 45% of the total cellular actin and ~70% of actin-binding protein remain in the detergent-insoluble cell residue. The soluble actin is not filamentous as determined by sedimentation analysis, the DNAase I inhibition assay, and electron microscopy, indicating that the cytoskeleton is not fragmented by detergent extraction. The spacing between the ramifications of the actin network is 94 ± 47 nm and 118 ± 72 nm in cytoskeletons prepared for electron microscopy by freeze drying and critical point drying, respectively. Free filament ends are rare, except for a few which project upward from the body of the network or which extend down to the substrate. Filaments of the network intersect predominantly at right angles to form either T-shaped and X-shaped overlaps having striking perpendicularity or else Y-shaped intersections composed of filaments intersecting at 120-130° angles. The actin filament concentration in the lamellae is high, with an average value of 12.5 mg/ml. The concentration was much more uniform in freeze-dried preparations than in critical point-dried specimens, indicating that there is less collapse associated with the freezing technique.

The orthogonal actin network of the macrophage cortical cytoplasm resembles actin gels made with actin-binding protein. Reaction of cell cytoskeletons and of an actin gel made with actin-binding protein with anti-actin-binding protein IgG and anti-IgG-coated gold beads resulted in the deposition of clusters of gold at points where filaments intersect and at the ends of filaments that may have been in contact with the membrane before its removal with detergent. In the actin gel made with actin-binding protein, 75% of actin-fiber intersections labeled, and the filament spacing between intersections was consistent with that predicted on theoretical grounds if each added actin-binding protein molecule cross-links two filaments to form an intersection in the gel. On the other hand, only 38% of all filament intersections in the macrophage cortical cytoskeletons labeled, and the filament spacing between junctions was much smaller than that predicted from the molar ratio of actin binding protein to actin in the cytoskeletons. Despite the differences between actin gels made with actin-binding protein and macrophage cytoskeletons, which presumably arise from the greater com-

1002319320

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

plexity of the cell compared with the purified protein assembly, the networks were qualitatively similar.

Hartwig, J. H. and Shevlin, P. (*Stossel, T.*)

The Journal of Cell Biology **103**:1007-1020; 1986.

Other support: National Institutes of Health, National Science Foundation and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston.

PROBING THE MECHANISM OF INCORPORATION OF FLUORESCENTLY LABELED ACTIN INTO STRESS FIBERS

The mechanism of actin incorporation into and association with stress fibers of 3T3 and WI38 fibroblasts was examined by fluorescent analog cytochemistry, fluorescence recovery after photobleaching (FRAP), image analysis, and immunoelectron microscopy. Microinjected, fluorescein-labeled actin (AF-actin) became associated with stress fibers as early as 5 min post-injection. There was no detectable cellular polarity in the association of AF-actin with pre-existing stress fibers relative to perinuclear or peripheral regions. The rate of incorporation was quantified by image analysis of images generated with a two-dimensional photon counting microchannel plate camera. After equilibration of up to 2 h post-injection, FRAP demonstrated that actin subunits exchanged rapidly between filaments in stress fibers and the surrounding cytoplasm. When co-injected with rhodamine-labeled bovine serum albumin as a control, only actin was detected in the phase-dense stress fibers. The control protein was excluded from fibers and any linear fluorescence of the control was demonstrated as a pathlength artifact. The incorporation of AF-actin into stress fibers was studied by immunoelectron microscopy using anti-fluorescein as the primary antibody and goat anti-rabbit IgG coupled to peroxidase as the secondary antibody. At 5 min post-injection, reaction product was localized periodically in some fibers with a periodicity of ~0.75 μm. In large diameter fibers at 5 min post-injection, the analog was seen first on the surface of fibers, with individual filaments resolvable within the core. In the same cell, thinner diameter fibers were labeled uniformly throughout the diameter. By 20 min post-injection, most fibers were uniformly labeled. We conclude that the rate of actin subunit exchange *in vivo* is extremely rapid with molecular incorporation into actin filaments of stress fibers occurring as early as a few minutes post-injection. Exchange appears to first occur in filaments along the surface of stress fibers and then into more central regions in a periodic manner. We suggest that the periodic localization of actin at very early time points is due to a local microheterogeneity in which microdomains of fast vs. slower incorporation result from the periodic localization of actin-binding protein, such as α-actinin, along the length of the fiber.

Amato, P. A. and *Taylor, D. L.*

The Journal of Cell Biology **102**:1074-1084, March 1986.

Other support: National Institutes of Health.

From the Department of Biological Sciences and Center for Fluorescence Research in Biomedical Sciences, Carnegie-Mellon University, Pittsburgh.

1002213371

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

angiotensin converting enzyme, carboxypeptidase N, and carbonic anyhydrase; enzyme inhibitors such as α_2-macroglobulin; receptors for bradykinin and other hormones; intracellular enzymes for synthesis of prostaglandins and for the synthesis of extracellular products such as components of the glycocalyx and basement membranes. Endothelial cells of the lungs play an active role in determining the quality of systemic arterial blood. Endothelial vesicles that open directly to the vascular space provide an ultrastructural specialization that is ideally suited for favoring interaction between solutes and colloids of plasma with endothelial cell-bound enzymes.

Ryan, U. S.

Progress in Applied Microcirculation **9**:150-164. 1985.

Other support: National Insitutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

HPLC ANALYSIS OF AMINO ACIDS WITH ION EXCHANGE CHROMATOGRAPHY AND O-PHTHALALDEHYDE POST-COLUMN DERIVATIZATION: APPLICATIONS TO THE ASSAY OF ENDOGENOUS FREE AMINO ACIDS AND THEIR TRANSPORT IN HUMAN PLACENTAL VILLUS

A method using high performance liquid chromatography (HPLC) for the analysis of primary amino acids in human placenta is described. This method involves separation of primary amino acids by high performance ion-exchange chromatography followed by post column derivatization using O-phthalaldehyde (OPA) and 2-mercaptoethanol and fluorescence (excitation 340 nm and emission 410 nm) detection of derivatives. Waters 840 HPLC Amino Acid System was used for this purpose. For analysis, villus tissue was extracted with acetonitrile, and the recovered amino acids were reconstituted in a sodium diluent (pH 2.2). The complete profile of the primary amino acids in the sample could be constructed in about 90 minutes. Up to 44 samples can be analyzed without special attention. Using this method, essential amino acids (threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine) and nonessential amino acids (aspartic acid, serine, glutamic acid, glycine, alanine, arginine) were detected and quantified in human placental villus in pmol quantities. Plots of peak heights (or areas) were linear for several amino acids. The same method was also used for (a) the assay of free primary amino acids in umbilical bloods, (b) the efflux of amino acids from isolated human placental villus, and (c) to study the uptake of α-aminoisobutyric acid (AIB), a non-metabolizable amino acid, by the isolated placental villus.

Sastry, B. V. R., et al.

Journal of Liquid Chromatography **9**(8):1689-1710, 1986.

Other support: National Institutes of Health.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

1002319317

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

HUMAN LEUKOCYTE CATHEPSIN G. SUBSITE MAPPING WITH 4-NITROANILIDES, CHEMICAL MODIFICATION, AND EFFECT OF POSSIBLE COFACTORS

The extended substrate binding site of cathepsin G from human leukocytes has been mapped by using a series of peptide 4-nitroanilide substrates. The enzyme has a significant preference for substrates with a P_1 Phe over those with the other aromatic amino acids Tyr and Trp. The S_2 subsite was mapped with the substrates Suc-Phe-AA-Phe-NA where AA was 13 of the 20 amino acid residues commonly found in proteins. The best residues were Pro and Met. The S_3 subsite was mapped with the sequence Suc-AA-Pro-Phe-NA by using 14 different amino acid residues for AA. The two best residues were the isosteric Val and Thr. No significant improvement in reactivity was obtained by extending the substrate to include seven different P_4 residues. The kinetic parameters for cathepsin G are significantly slower than those for many other serine proteases. Changes in the reaction conditions and addition of possible cofactors or ligands were in general found to have little effect on the enzymatic activity, while chemical modifications and proteolysis destroyed the activity of cathepsin G. Cathepsin G hydrolyzed peptides containing model desmosine residues and prefers the hydrophobic picolinoyllysine derivative over lysine by substantial margins at both the S_1 and S_2 subsites but will not tolerate it at S_3. Substrates with sequences related to the cathepsin G cleavage site in angiotensin I and angiotensinogen, and the reactive site of α_1-antichymotrypsin, were hydrolyzed effectively by enzyme, but with unexceptional rates. Our results indicate that the natural substrate(s) and function(s) of cathepsin G still remain to be discovered.

Tanaka, T., Minematsu, Y., Reilly, C. F., and *Travis, J.*

Biochemistry **24**:2040-2047, 1985.

From the Department of Biochemistry, University of Georgia, Athens.

INHIBITION OF HUMAN LEUKOCYTE ELASTASE, CATHEPSIN G, CHYMOTRYPSIN A_α, AND PORCINE PANCREATIC ELASTASE WITH SUBSTITUTED ISOBENZOFURANONES AND BENZOPYRADIONES

Several 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones, 3-(1-haloalkylidene)-1(3*H*)-isobenzofuranones, and 3-bromomethyl-1*H*-2-benzopyran-1-ones containing masked halo ketone functional groups were synthesized and tested as inhibitors of several serine proteases including human leukocyte (HL) elastase and cathepsin G. While many of the 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones were quite potent inhibitors of the enzymes tested, the alkylideneisobenzofuranones and benzopyran-1-ones inhibited poorly or not at all. The 3-halo-3-(1-haloalkyl)-1(3*H*)-isobenzofuranones decomposed rapidly upon addition to buffer to give the corresponding 3-alkyl-1*H*-2-benzopyran-1,4(3*H*)-diones. The pure benzopyran-1,4-diones were extremely potent inhibitors of HL elastase and chymotrypsin A_α but did not inactivate porcine pancreatic elastase or cathepsin G. Enzymes inhibited by the isobenzofuranones and benzopyran-1,4-diones regained activity slowly upon standing or after dialysis ($t_{1/2}$ = 5–16 h) and more rapidly in the presence of 0.5 M hydroxylamine, which indicated the presence of labile acyl moieties in the inhibited enzyme. These results are consistent with a scheme in which the active site serine of the protease reacts with the lactone carbonyl of these inhibitors to give a stable acyl enzyme and alkylation

1002319324

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

of another active site residue by the unmasked halo ketone functional group does not occur.

Hemmi, K., Harper, J. W., and Powers, J. C. (*Travis, J.*)

Biochemistry **24**:1841-1848, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

MAMMALIAN CHYMOTRYPSIN-LIKE ENZYMES. COMPARATIVE REACTIVITIES OF RAT MAST CELL PROTEASES, HUMAN AND DOG SKIN CHYMASES, AND HUMAN CATHEPSIN G WITH PEPTIDE 4-NITROANILIDE SUBSTRATES AND WITH PEPTIDE CHLOROMETHYL KETONE AND SULFONYL FLUORIDE INHIBITORS

The extended substrate binding sites of several chymotrypsin-like serine proteases, including rat mast cell proteases I and II (RMCP I and II, respectively) and human and dog skin chymases, have been investigated by using peptide 4-nitroanilide substrates. In general, these enzymes preferred a P_1 Phe residue and hydrophobic amino acid residues in P_2 and P_3. A P_2 Pro residue was also found to be quite acceptable. The S_4 subsites of these enzymes are less restrictive than the other subsites investigated. The substrate specificity of these enzymes was also investigated by using substrates which contain model desmosine residues and peptides with amino acid sequences of the physiologically important substrates angiotensin I and angiotensinogen and α_1-antichymotrypsin, the major plasma inhibitor for chymotrypsin-like enzymes. These substrates were less reactive than the most reactive tripeptide reported here, Suc-Val-Pro-Phe-NA. The thiobenzyl ester Suc-Val-Pro-Phe-SBzl was found to be an extremely reactive substrate for the enzymes tested and was 6–171-fold more reactive than the 4-nitroanilide substrate. The four chymotrypsin-like enzymes were inhibited by chymostatin and N-substituted saccharin derivatives which had K_i values in the micromolar range. In addition, several potent peptide chloromethyl ketone and substituted benzenesulfonyl fluoride irreversible inhibitors for these enzymes were discovered. The most potent sulfonyl fluoride inhibitor for RMCP I, RMCP II, and human skin chymase, 2-(Z-NHCH$_2$CONH)C$_6$H$_3$SO$_2$F, had $k_{obs}/[I]$ values of 2500, 270, and 1800 $M^{-1}s^{-1}$, respectively. The substrates and inhibitors reported here should be extremely useful in elucidating the physiological roles of these proteases.

Powers, J. C., Tanaka, T., Harper, J. W., and Minematsu, Y. (*Travis, J.*)

Biochemistry **24**:2048-2058, 1985.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

REACTION OF SERINE PROTEASES WITH SUBSTITUTED ISOCOUMARINS: DISCOVERY OF 3,4-DICHLOROISOCOUMARIN, A NEW GENERAL MECHANISM BASED SERINE PROTEASE INHIBITOR

The mechanism-based inactivations of a number of serine proteases, including human leukocyte (HL) elastase, cathepsin G, rat mast cell proteases I and II, several

1002319325

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000

THE MECHANISMS OF THE INACTIVATION OF HUMAN ALPHA-1-PROTEINASE INHIBITOR BY GAS-PHASE CIGARETTE SMOKE

Cigarette smoke, either directly or indirectly, causes alpha-1-proteinase inhibitor (αIPI) to lose elastase inhibitory capacity (EIC), leaving lung connective tissues susceptible to proteolytic degradation. This paper discusses possible mechanisms for inactivation by cigarette smoke (CS) and by a model system [NO, isoprene, and air] that duplicates much of CS free radical chemistry. Inactivation of αIPI by either CS or the model is biphasic, a fast inactivation is followed by a slower one. With pre-prepared extracts, only the slow inactivation is observed. Apparently short-lived species in the smoke itself and the model system cause the fast inactivation; they may be peroxynitrates, which form in smoke from nitrogen dioxide and peroxyl radicals. The slower inactivation appears to involve hydrogen peroxide and/or organic hydroperoxides or species produced by them. Incubation of αIPI with linoleic acid produces a slow loss of EIC, prevented by the presence of vitamin E, which supports the hypothesis of a route involving lipid hydroperoxides. Protection of αIPI by various types of compounds shows that unprotonated amines and amino acids protect, but the protonated or acylated compounds do not. Ascorbate and glutathione provide the strongest protection.

Pryor, W. A., et al.

Advances in Free Radical Biology & Medicine 2:161-188, 1986.

Other support: National Institutes of Health and the National Foundation for Cancer Research.

From the Biodynamics Institute, Louisiana State University, Baton Rouge.

USE OF ENDOTHELIAL CELLS IN CULTURE FOR STUDIES OF THE MICROCIRCULATION

Physiological studies have indicated that endothelial cells, especially those of the lungs, play an active role in the processing of blood-borne vasoactive substances, including adenine nucleotides, biogenic amines and polypeptide hormones. Studies using cultures of pure lines of pulmonary endothelial cells confirm the hypothesis that circulating ATP, ADP, and AMP are metabolized by enzymes on the luminal surface of endothelial cells. Cytochemical localization at the electron microscope level shows that the enzymes responsible are located in those endothelial caveolae that directly face the vascular lumen. Likewise, pure cultures of endothelial cells metabolize bradykinin and angiotensin I to yield the same products as do intact lungs. Immunocytochemical localization at the electron microscope level indicates that angiotensin converting enzyme (EC 3.4.15.1), the enzyme responsible for both degradation of bradykinin and conversion of angiotensin I to angiotensin II, is located on both caveolae and on undifferentiated portions of the endothelial plasma membrane. The enzyme is located with its active site directly accessible to plasma substrates. Endothelial cells in culture provide a means for studying endothelial properties without the complication of enzymic contributions from other cell types. For example, endothelial cells possess, and probably synthesize, surface enzymes such as ATPase, ADPase, 5'-nucleotidase,

1002319316

Source: https://www.industrydocuments.ucsf.edu/docs/jqxk0000